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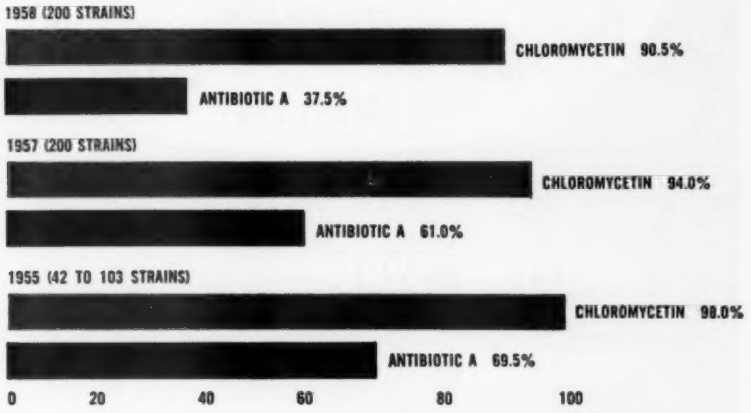

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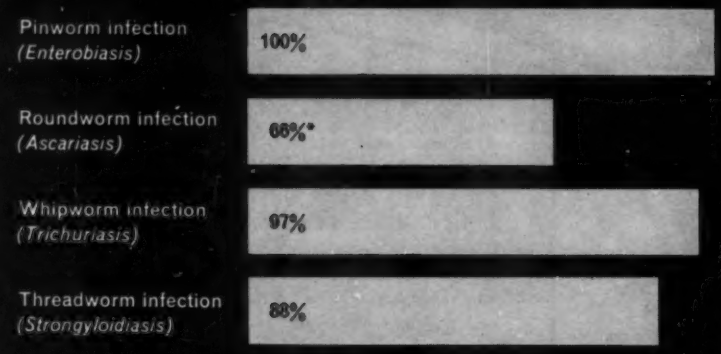

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ORGANOPHOSPHORUS COMPOUNDS AS RESIDUAL TREATMENTS FOR ADULT MOSQUITO CONTROL[1]

W. MATHIS AND H. F. SCHOOF

Resistance to DDT or dieldrin residual applications by malaria vectors throughout the world is a handicap of increasing importance to malaria control and eradication programs. Because of this situation, studies to evaluate other insecticides as potential residual treatments against anopheline vectors were included in a contractual agreement between the Communicable Disease Center and the International Cooperation Administration.

Preliminary laboratory tests of various commercial pesticides[2] on plywood panels against susceptible adult female *Anopheles quadrimaculatus* at exposure periods of 30 minutes indicated that at 100 mg/sq. ft., Parathion, Malathion, DDT, EPN, endrin, and dieldrin were the superior toxicants, giving satisfactory kills for extended periods. In general, residues from suspensions were superior to those from emulsions, only dieldrin and Parathion being effective with either type of formulation.

In subsequent laboratory tests, dosages of EPN suspensions at 12.5, 25, or 50 mg/sq. ft., combined with 100 mg of DDT/sq. ft. were superior to either compound used alone at these dosages. Malathion suspensions at dosages of 25, 50, or 100 mg/sq. ft., alone, were effective only at the highest dosage. Similar dosages of Malathion in combination with 100 mg of DDT/sq. ft. gave satisfactory kills only at levels of 50 or 100 mg of Malathion/sq. ft., while Parathion at dosages of 12.5, 25, or 50 mg/sq. ft. was effective at the two higher dosages. In combination with 100 mg of DDT/sq. ft., all three dosages of Parathion gave satisfactory results. Against field-collected dieldrin-resistant *A. quadrimaculatus* (Mathis *et al.*, 1956), satisfactory results were obtained with suspensions of Parathion at dosages of 12.5, 25, and 50 mg/sq. ft.; of Malathion at 100 mg/sq. ft.; and of EPN at 50 mg/sq. ft. Dosages of EPN at 12.5 and 25 mg/sq. ft. and of Malathion at 12.5, 25, and 50 mg/sq. ft. produced unsatisfactory results. Residues which produced mortalities of at least 90 per cent with 30-minute exposures for at least 10 weeks were considered as "satisfactory."

As a result of these laboratory evaluations, further tests with Malathion, Parathion, and EPN were conducted under simulated field conditions using dieldrin-resistant and susceptible strains of *A. quadrimaculatus*.

METHODS

In 1956, two shed-type huts, approximately 8 feet × 8 feet × 8 feet, were constructed in a wooded area adjacent to irrigated rice fields near Savannah, Georgia. Under the rear overhang of the roof, an opening (1 inch) was left so that wild mosquitoes could enter. On either side, a window (3 feet × 3 feet) was constructed with V-shaped louvers equipped with 1-inch openings in such a manner that mosquitoes could readily leave the hut but would have difficulty in re-entering it. Screen traps were placed over the window to capture the mosquitoes leaving the hut. The inside of the hut was fitted with removable plywood panels which were sprayed with water-wettable Malathion at the rate of 100 mg/sq. ft. Two domestic rabbits, placed in each hut prior to operation at night, served as an attractant and a source of blood.

Evaluations were made at weekly intervals for 5 weeks after treatment using the natural population of *A. quadrimaculatus*. The mosquitoes entering the huts during the night were collected from the interior of the huts or the window traps the following morning by 9:00 a.m. After the numbers of mosquitoes entering the hut were insufficient for satisfactory tests, 100 laboratory-reared female *A. quadrimaculatus* were released at weekly intervals in the hut and left for 1 hour. After this exposure, they were

[1] From the Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia.

[2] Compounds tested included aldrin, American Cyanamid Compound 4124, Bayer 21/199, BHC, chlordane, Chlorthion, DDT, Diazinon, dieldrin, Dipterex, endrin, EPN, heptachlor, isodrin, lindane, Malathion, methoxychlor, Parathion, Perthane, Strobane, TDE, and toxaphene.

collected, fed honey water, and held for 24 hours at 80° F and 70 per cent relative humidity for mortality determination.

In 1957, similarly equipped huts (6 feet × 6 feet × 6 feet) with smaller windows (2 feet × 2 feet) were used. As laboratory-reared mosquitoes were employed, an entrance cage (2 feet × 1 feet × 1 feet) was attached to the rear of the hut at window level (2 feet above floor) with an opening (1 inch × 20 inches) to permit the mosquitoes to enter the hut. The removable plywood panels were treated prior to installation as follows: (a) DDT (200 mg/sq. ft.), (b) dieldrin (25 or 50 mg/sq. ft.), (c) Malathion (100 or 200 mg/sq. ft.), (d) Malathion: DDT (200:100 or 100:200 mg/sq. ft.), (e) Malathion:dieldrin (100:25 or 100:50 mg/sq. ft.), (f) Parathion (12.5 mg/sq. ft.), (g) Parathion: DDT (12.5:200 mg/sq. ft.), and (h) EPN:DDT (25:200 mg/sq. ft.). All applications were made at 40 psi with a hand sprayer equipped with an "8004" nozzle, certain of the higher dosages being applied prior to the tests and the panels stored in a well-ventilated room for aging. With the panels installed, all the inner resting surfaces of the hut were treated except for the floor and the window louvers. After each test, the treated panels were removed and placed in storage. The huts were located in a shaded, woody area in close proximity to each other. Temperatures ranged from 62° to 91° F during the test periods.

Each test began at 4:00 p.m., when one domestic rabbit was placed in the hut and the mosquitoes were transferred to the entrance cage. At that time, the entrance slot at the rear was opened and remained so until the following morning, when it was closed, the rabbit removed, and the dead and living mosquitoes collected from the hut and window traps. The living mosquitoes were placed in screen holding cages, fed, and held for a 24-hour mortality determination.

Three-day-old laboratory-reared females of a dieldrin-resistant strain and of a susceptible strain of *A. quadrimaculatus* were used in the tests, the former being obtained from eggs from field-collected females in Mississippi. Tests were made with each formulation against the dieldrin-resistant strain at 2-week intervals and against the susceptible strain as time permitted. Approximately 50 to 150 females were used in each test, the number available fluctuating because of seasonal variations in the natural abundance of the wild mosquitoes. Experiments were conducted with the dieldrin-resistant strain from June 25 to October 3. For comparative purposes, one untreated hut was operated during each test night.

RESULTS

The 1956 application of Malathion (100 mg/sq. ft.) gave essentially 100 per cent mortality of the wild *A. quadrimaculatus* recovered within the hut for a period of 5 weeks after treatment. In the window traps, the mortality range was from 65 to 100 per cent. The lowest mortality occurred with the first run when 559 females were recovered in the window trap. Based on total numbers recovered in the treated and untreated huts, the Malathion treatment apparently did not repel this strain of mosquito, since a greater number was recovered in the treated than in the untreated hut. The percentage of females obtaining blood meals ranged from 7 to 22 per cent in the untreated hut and from 3 to 15 in the treated hut. Only two of the blood-fed mosquitoes from the treated hut survived for 24 hours after exposure.

The laboratory strain of *A. quadrimaculatus* females used in tests 7, 8, 9, 10, and 13 weeks after treatment gave 24-hour mortalities of 100, 98, 100, 70, and 36 per cent, respectively, with a 60-minute exposure period.

Table 1 gives the monthly mortalities ob-

TABLE 1

Average 24-hour mortalities obtained with dieldrin-resistant Anopheles quadrimaculatus *females in treated animal bait stations (June–October, 1957)*

Toxicant (water-wettable)	Dosage (mg/sq. ft.)	Per cent mortality at months after treatment				
		1	2	3	4	5
DDT	200	—	30	26	54	54
Dieldrin	50	51	47	41	46	
Dieldrin	25	75	36	40		
Malathion	200	—	100	100	100	100
Malathion	100	100	99	100	100	
Malathion:DDT	100:200	—	100	100	100	
Malathion:DDT	200:100	—	100	100	100	
Malathion:dieldrin	100:50	100	100	100	100	
Malathion:dieldrin	100:25	100	100	99		
Parathion	12.5	100	100	100		
Parathion:DDT	12.5:200	—	100	95	99	100
EPN:DDT	25:200	—	96	88	84	

tained in the 1957 tests with dieldrin-resistant *A. quadrimaculatus* females. Although specific exposure times are unknown, limited observations indicate that most mosquitoes entered the hut around 7:00 p.m. These specimens then were collected between 8:00 and 10:00 a.m. the following morning. All formulations containing organic phosphorus compounds alone or in combination with dieldrin or DDT, with the exception of EPN:DDT, gave essentially 100 per cent mortality for the duration of the test (11 to 20 weeks). The EPN:DDT formulation, which was not evaluated during the first 4 weeks after treatment, showed mortalities of 98 to 100 per cent during the following 12 weeks with mosquitoes recovered in the house, but kills in the window traps varied from 27 to 100 per cent. However, this combination still was superior to DDT alone, although inferior to formulations containing Malathion or Parathion. Dieldrin (25 or 50 mg/sq. ft.) gave mortalities of 40 to 75 per cent, while DDT (200 mg/sq. ft.) evaluated the second, third, fourth, and fifth months after treatment, yielded mortalities of only 30, 26, 54, and 54 per cent, respectively.

Against the susceptible laboratory strain, all formulations with the exception of DDT gave essentially 100 per cent mortality. During the first 3 months of exposure to DDT, the mortalities of the laboratory strain were higher than those of the dieldrin-resistant strain but little difference was noted during the fourth and fifth months.

The number of females recovered and the mortality occurring in the entrance cage with the various treatments were compared with the same information from the entrance cages attached to the untreated huts. With all treatments, a greater percentage of females remained in the entrance cage than in cages of untreated huts for corresponding nights. Although the numbers recovered varied greatly, no one formulation seemed to repel consistently more than the others.

When DDT, dieldrin, or EPN plus DDT was used as the treatment, the entrance cage mortality was no greater than that of the untreated hut. All other treatments apparently caused considerable mortality in the entrance cage. Malathion in combination with DDT or dieldrin caused the greatest mortality in the entrance cages, in some instances all mosquitoes remaining in the entrance cage were killed. Mortality appeared to be greater on nights of very high humidity.

In the untreated hut, the total mortalities, with few exceptions, were less than 10 per cent. Comparisons were made on the behavior of the two strains under untreated conditions. Of the 3,700 females of the dieldrin-resistant strain introduced into the entrance cage, 80 per cent entered the house and of these 58 per cent remained until collected the following morning. Only five females were noted with blood meals. With the susceptible laboratory strain, 85 per cent of the 1,345 females introduced entered the house and 88 per cent of this number remained until collected the following morning. Twelve per cent of the number entering the house obtained a blood meal. Thus, the percentage of mosquitoes that entered the house was essentially the same with the two strains. However, the number which remained in the house varied 30 percentage points in favor of the laboratory strain but since the dieldrin-resistant strain showed a lesser inclination to feed on the rabbit, the lack of an attractive host may have influenced their behavior.

The lowered mortality rates obtained with certain treatments was partially a reflection of the escape response which presumably reduced the exposure period of specimens recovered in the window traps. With DDT, the range in mortality of dieldrin-resistant specimens collected in the hut was between 25 and 71 per cent, with dieldrin between 53 and 70 per cent, and with EPN:DDT between 98 and 100 per cent. In the window trap, the per cent mortalities for the same three treatments were 16 to 41, 22 to 41, and 45 to 86, respectively. However, in all treatments with Malathion (alone or combined with DDT or dieldrin) or with Parathion, 98 to 100 per cent mortalities were obtained regardless of the site of collection. The data for Malathion and Parathion presumably are the result of the greater toxicity of these insecticides to the mosquitoes, since the average percentages of mosquitoes which entered the window traps with the DDT, dieldrin, Malathion, or Parathion treatments fell in the range of 36 to 49 per cent. Contrary to expectation, the percentages of mosquitoes for the DDT or dieldrin applications were essentially the same (46 versus 49%). Against the susceptible strain, window trap and hut mortalities with all compounds

were in the same general range, the majority of the specimens being recovered in the hut.

DISCUSSION

These results indicate Malathion residues to have a much longer activity against mosquitoes than would be assumed from the short-term effectiveness this toxicant exhibits against house flies. Although the residues on the plywood panels in the huts did not receive the type of exposure to air and dust encountered in an inhabited dwelling, the biweekly removal, storage, and reinstallation of the panels subjected the deposits to handling abuse. The influence of occupied dwellings on the residual action of Malathion residues should be evaluated in future studies.

Of particular interest were the essentially complete kills achieved with Malathion and Parathion against the dieldrin-resistant strain as compared with the 26 to 54 per cent mortalities obtained with DDT. Also of interest was the fact that the dieldrin-resistant strain succumbed to dieldrin residues at mortality levels similar to those of DDT.

SUMMARY

The residual action of Malathion and Parathion deposits against laboratory-reared adult *Anopheles quadrimaculatus* was evaluated in animal-baited huts under simulated field conditions near Savannah, Georgia. Malathion at 100 and 200 mg/sq. ft., Malathion:DDT at 100:200 or 200:100 mg/sq. ft., Malathion:dieldrin at 100:25 or 100:50 mg/sq. ft., Parathion at 12.5 mg/sq. ft. and Parathion:DDT at 12.5:200 mg/sq. ft. produced essentially 100 per cent mortalities of dieldrin-resistant females for the entire 4- to 5-month observation period. Dieldrin at 25 or 50 mg/sq. ft. and DDT at 200 mg/sq. ft. yielded mortalities of 26 to 54 and 36 to 75%, respectively, over the same time interval. Dieldrin-resistant and susceptible strains of *A. quadrimaculatus* moved into untreated huts from attached entrance cages in similar numbers but 42 per cent of the resistant specimens escaped into window traps as compared to 12 per cent of the susceptible strain.

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FEEDING HABITS OF SYMBIOTIC MESOSTIGMATID MITES OF MAMMALS IN RELATION TO PATHOGEN-VECTOR POTENTIALS[1]

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Most arthropod-borne pathogens occurring in the circulatory system of vertebrate hosts are transmitted by vectors having an obligatory, skin-piercing, food relationship with their hosts. But the mere presence of presumably parasitic arthropods, such as mites, in nests and on vertebrates is insufficient evidence of such an obligatory relationship. Information on food relationships must also be taken into account in assessing the pathogen-vector potentials of the mites.

With the exception of the known bloodsucking mites of the family Dermanyssidae, relatively little evidence has been reported incriminating mesostigmatid mites as vectors of pathogens. It has been assumed that they may be of considerable importance in the maintenance of enzootic pathogens which on occasion may be transmitted to man by mites or by other means. A case in point is that of the virus of hemorrhagic fever, believed by some to be transmitted by *Laelaps jettmari* Vitzthum. Traub *et al.*, (1954) point out that this has not been proved, and that the mites apparently do not feed on the blood of the rodents from which they are collected, nor are they known to bite man.

As early as 1908, Miller demonstrated the development of *Hepatozoon perniciosum* (now *H. muris*) in *Echinolaelaps echidninus* (Berlese) with transmission to the rat host through ingestion of the infective mites. Clark (1958) demonstrated development of the closely related *Hepatozoon griseisciuri* of the eastern grey squirrel in *E. echidninus* and also in *Haemogamasus ambulans* (Thorell).

Since *Hepatozoon* is transmitted through the ingestion of infected mites by vertebrate hosts, it may be unnecessary for the mite vectors to have an obligatory, skin-piercing, bloodsucking habit. In this sense the mites may represent an exception to the rule, for if they are general feeders, ingesting blood opportunistically, they still may be of epizootological significance in transmitting a pathogen such as *Hepatozoon*.

Bregatova and Kolpakova (1952) threw suspicion on *Laelaps muris* (Ljungh), *Laelaps amphibius* (Zachvatkin), and *Haemolaelaps glasgowi* (Ewing) in connection with tularemia, pointing out that these were the most common mites on the water vole, *Arvicola terrestris*, the principal reservoir of tularemia in Russia. Subsequently, Ramonova *et al.*, (1955) reported the isolation of *Pasturella tularensis* from naturally infected *L. muris* and *H. glasgowi*. The significance of this finding depends upon the usual food habits of the mites concerned.

With the exception of records of the skin-piercing and bloodsucking propensities of dermanyssid mites, such as the tropical rat mite, *Ornithonyssus bacoti* (Hirst), relatively few observations have been recorded for related families containing parasitic species. On the basis of mite association with vertebrates, plus the presence of blood in the mites' intestinal tracts, the assumption has been that these organisms pierce the skin of the host and thus obtain nourishment. This is not in accord with the fact that the mouthparts of many of the laelaptids and haemogamasids concerned do not appear to be particularly well adapted for a piercing function. In contrast to the sharply pointed, untoothed chelicerae of the dermanyssid mites (proved skin penetrators) the chelicerae of laelaptids are usually more generalized in structure. The paired digits of most laelaptid and haemogamasid chelicerae, although frequently highly modified in the male for transference of the sperm, are otherwise toothed and adapted for grasping and tearing, and in some cases for rasping.

Reports involving artificial culture of parasitic laelaptids indicate that several species feed readily on free blood or serum. Skaliy and Hays (1949) fed *E. echidninus* on a free pool of rat blood, but obtained no feeding through membranes. Owen (1956) fed the same species human

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blood through a piece of silk bolting cloth. He also observed cannibalism in females which attacked larvae. Grokhovskaya (1955) observed *H. glasgowi* feeding on man. However, Wharton and Cross (1957) reported that *E. echidninus* and *Laelaps nuttalli* Hirst would not feed through the intact skin of the rat, chick, or human, nor would *H. glasgowi* feed through the intact skin of man. All three species of mites fed readily on blood offered through fine-mesh bolting cloth. The authors postulated that the mites differ from many ectoparasites in that they do not regularly penetrate the unbroken skin of their hosts, but depend upon other ectoparasites, accidents, or the scratching of the host itself to prepare the skin for them.

Eubrachylaelaps circularis (Ewing) has been fed on free blood by Allred (1957a). The same investigator (1957b), working with the haemogamasid mite, *Brevisterna utahensis* (Ewing), indicated that the mite was able to penetrate the skin of its host *Neotoma lepida lepida*, the desert wood rat. Allred states (*in litt.*), however, that feeding tests were conducted after epidermal scales were carefully scraped from the tail of the rat host. Although efforts were made to avoid injuring the skin by this procedure, some doubt remains that the skin was unbroken prior to feeding by the mites. Here, however, as in the case of a few other haemogamasid mites, the mouthparts have departed from the typical laelaptid type to such a degree that it is less difficult to envisage their use in skin penetration.

MATERIALS AND METHODS

In the investigations reported here, the greatest emphasis was placed upon *Haemogmasus ambulans* (Thorell). This mite has been reported from a wide variety of hosts in Europe and North America. In the area around Berkeley, California, it occurs commonly in nests of *Neotoma fuscipes*, the dusky-footed wood rat. It is also found frequently in nests of *Microtus californicus*. In lesser numbers it occurs on the bodies of these hosts.

Haemogamasus liponyssoides Ewing was obtained for culture and feeding tests from the nests and bodies of *Microtus californicus*, the usual host of the mite in the San Francisco Bay area. *Haemogamasus oudemansi* Hirst was obtained from nest material of *N. fuscipes*, often in association with the more frequently encountered *H. ambulans*. *E. echidninus* and *O. bacoti* were obtained from *Rattus norvegicus*, which inhabits dumps in the San Francisco Bay area. *H. glasgowi*, which occurs on a wide range of rodent hosts, was collected for feeding studies from nests of *N. fuscipes*. *Brevisterna utahensis* was collected as a regular inhabitant of nests of *Neotoma lepida* in arid areas of southern California, and as an occasional inhabitant of nests of *N. fuscipes* in northern California.

Mites were maintained and fed, except as otherwise indicated, in small, glass, culture tubes approximately 7 mm in diameter and 5 to 8 cm in length, stoppered with cotton plugs at either end. In the majority of tests, the tubes were held in a humidity chamber on a wire grid over moist sand, at room temperatures usually ranging from 60° to 70° F. The relative humidity of the chamber was over 90 per cent. Except as otherwise indicated, mites were fed daily by supplying a drop of heparinized laboratory-mouse blood on a clean, cotton stopper.

Feeding trials were made on the intact skin of various adult rodents under Nembutal sedation, by applying the open end of a vial containing a known number of mites (usually 10 or less) to various areas of the host skin, such as the abdomen, genital area, ear, and axillae. Exposure periods varied from 20 to 90 minutes.

Feeding trials with the vial technique described above were also made on various hosts after the epidermis was gently scraped until serous exudate or blood appeared.

Feeding and culture trials with mites on suckling mice were made by the method of Radovsky (1958), designated as the free-access technique. Unrestrained mites were placed on series of the hosts for periods ranging up to 3 weeks. The mice were held in small plastic boxes measuring 4 inches square and 2.5 inches high, with a 3-inch-square section of the plastic lids replaced by 84-mesh nylon cloth. Included in each box were four moistened cotton balls about 1 inch in diameter, and autoclaved *Microtus* nest material to a depth of 1 inch. This material was maintained in a moist condition. The boxes were kept in the humidity chambers previously described. Mice were changed in each box at 24- to 48-hour intervals.

Dried blood for feeding trials was prepared from whole blood obtained by heart puncture of laboratory mice. The blood was dried at 98° F,

and ground into granules approximately 1 mm in diameter.

Feeding trials on flea feces were made by cementing a single thickness of voile to form a partition between two lengths of plastic tubing. Starved mites were held on one side of the partition, with freshly engorged *Xenopsylla cheopis* (Rothschild) on the other. Flea feces deposited on the voile partition were available to the mites.

The ability of mites to survive and reproduce on waste food of the host plus host feces and urine was investigated by placing a layer of plaster of Paris in the bottom of a glass jar. On this was placed sterilized nest material to which was added a pure culture of a single mite species. The host rodent was suspended in a wire cage at the top of the jar. A band of Ortho "Dead-line," serving as a mite barrier, was placed on the inside of the jar below a rubber friction band which in turn was cemented near the top of the jar to keep cage debris from contacting the "Dead-line." Such a culture could be held for 2 weeks or longer, between transfers to clean containers, and allowed the mites access to waste products of the host without access to the host itself. A simpler device was used for the same purpose in later tests. It consisted in a large, shallow tray containing water. Supported on the tray by large corks extending above the water was a second shallow tray slightly smaller than the first. In it were placed sterilized nest material and mites. Supported above the two trays by heavy wire legs resting on the table surface was a wire mesh cage containing a *Microtus*. All excess food, feces and urine fell into the tray containing mites. "Dead-line" barriers on the wire legs of the rodent cage prevented any stray arthropods from access to the assembly.

Predacious tendencies of mites were investigated by placing various species of arthropods in tubes with the mites for direct observation by stereoscopic microscopy. Except as indicated, such arthropods were normal, uninjured specimens.

RESULTS

Results of mite-feeding tests are summarized in Table 1. *Haemogamasus ambulans* fed readily on heparinized blood of laboratory mice. This food source was used routinely in maintaining laboratory cultures of the mite. Reproduction through several generations was successful, as reported elsewhere in detail (to be published). As a rule, deutonymphs and adults of *H. ambulans* fed immediately on liquid blood, engorging within 30 seconds. Larvae and protonymphs did not require food. Temperatures over a range of 36° to 75° F showed little or no effect on feeding. That the mites were not particular about the source of their blood meals was shown by their ready acceptance of free blood of the domestic pigeon and of the lizard *Gerrhonotus multicarinatus multicarinatus*.

Attempts to feed *H. ambulans* on hosts with intact skin produced essentially negative results. Two of a total of 40 mites applied by the vial technique to laboratory mice showed evidence of feeding, but a small abrasion found in the area to which these mites were applied is believed to have provided the food. Utilizing the same technique, subsequent attempts to feed this species through intact skin were uniformly unsuccessful, including trials on *N. fuscipes*, *Peromyscus californicus*, *P. truei*, and man. The mites did not seem attracted to any of these hosts. Their usual reaction upon contacting the skin was to run up the vial wall to the distal end. Placing a dark cloth over the vial did not change this response.

In an attempt to provide a more normal environment for feeding *H. ambulans* through intact skin, two sets of experiments were conducted, using the previously described free-access technique. The technique of the second set of experiments was varied by using 4- to 5-day-old mice in which umbilical scabs were absent, and by sealing the anal and genital areas with adhesive tape. Over the 2-week observation periods, the suckling mice were changed at 24 to 48 hour intervals. In the first experiment results with 11 adult test mites of both sexes were compared with those from 11 controls, similarly maintained, but not provided with a food source. Judging from their slightly darker appearance, as compared with the controls, the test mites seemed to have fed to some extent on the mice. Some reproduction also took place, resulting in four nymphs and one larva at the end of 2 weeks. The controls produced no young. Examination of the suckling mice revealed no evidence of punctures which might be attributable to the mites, but excoriations of the umbilical scabs on some were typical of the marks produced by mites feeding on dried blood, as described below. The second experi-

ment was run with 24 adult test mites and six controls of both sexes. At the end of 2 weeks only 13 adults and two nymphs remained in the test culture, and only two adults in the control. Whereas no evidence of skin lesions was found on any of the suckling mice used, three adult mites and one nymph from the test culture contained bright red blood at the termination of the experiment.

An interesting reaction, considered a questing response, was observed when the containers holding control mites were opened. The mites came to the surface of the nest material and climbed to the ends of the dried grass. Test mites

TABLE 1

Food sources utilized by mites

Food source	*H. ambulans*		*H. liponysoides*		*H. oudemansi*		*B. utahensis*		*E. echidinus*		*H. glasgowi*		*O. bacoti*	
	No. tested	% feeding	No. tested	% feeding	No. tested	% feeding	No. tested	% feeding	No. tested	% feeding	No. tested	% feeding	No. tested	% feeding
Heparinized blood of laboratory mouse	500	100	4	50	38	63	>75	>50	5	100	5	100	—	—
Penetration of intact skin of living host (vial technique)														
Neotoma	30	0	5	0	13	0	45	0	25	0	51	?	40	25
Peromyscus	12	0	—	—	—	—	—	—	—	—	—	—	—	—
Microtus	—	—	15	0	7	0	27	0	5	0	10	0	14	>80
Lab. rat	11	0	—	—	5	0	10	0	5	0	—	—	31	3
Lab. mouse	40	5	11	0	5	0	—	—	2	0	2	0	10	60
Man	20	0	—	—	7	0	20	—	—	—	20	?	20	0
Penetration of intact skin of suckling host. (free access for 1.5–2.5 hours)														
Microtus	—	—	8	12	—	—	—	—	—	—	—	—	—	—
Lab. mouse	5	0	6	50	—	—	10	0	10	0	—	—	—	—
Abraded skin of living host.														
Neotoma	14	7	5	100	7	0	10	0	20	55	10	100	10	50
Microtus	—	—	15	40	—	—	—	—	5	0	10	20	—	—
Lab. rat	—	—	—	—	—	—	—	—	—	—	18	84	10	100
Lab. mouse	8	0	—	—	5	0	—	—	5	100	—	—	—	—
Man	16	100	—	—	7	0	10	80	5	20	10	100	10	80
Dried blood of laboratory mouse	7	100	5	0	20	0	31	100	5	100	5	100	—	—
Flea feces	7	100	—	—	—	—	—	—	—	—	—	—	—	—
Intact, living arthropods														
Anoplura	10	100	5	0	—	—	—	—	5	100	3	100	—	—
Corrodentia	5	100	—	—	—	—	—		—	—	—	—	—	—
Collembola	5	100	—	—	—	—	—	—	—	—	—	—	—	—
Siphonaptera (larvae)	7	0	—	—	—	—	—	—	—	—	—	—	—	—

held with suckling mice remained hidden in the nest material when the containers were opened.

When the skin of a *Neotoma* was abraded, one of 14 *H. ambulans* adults fed on the resulting serous exudate; all of 16 specimens engorged when placed on the abraded skin of man. These results were obtained, using the vial feeding technique, with mites which had been starved for 24 to 72 hours. Detailed observations on six female and one male *H. ambulans* showed that they would feed readily on particles of dried, laboratory-mouse blood. They manipulated the particles with the chelae and pedipalpi, exuding a clear, salivary fluid which dissolved the blood as the chelae scraped and gouged the moistened area. The resultant liquid was then ingested, turning the gut a dark reddish-brown color. A culture of *H. ambulans* was subsequently maintained successfully for several weeks on a diet consisting solely of dried blood. Reproduction rates were almost as high as when the mites were fed on liquid blood. *H. ambulans* also was observed to feed readily on flea feces. The mites refused to feed on mouse feces, urine, sugar solution, or decaying plant material, in small-vial tests. Similarly, no evidence of successful feeding was seen in a mass culture started with 50 female and male mites, provided with waste food, feces and urine of *Microtus* by the method described earlier. After 2 weeks only 27 mites were recovered, the identical number surviving in a control culture of 50 mites which had no access to feces or urine of *Microtus*. By contrast, a test culture of *H. ambulans* similar to the above, but contaminated by tyroglyphid mites, more than doubled in population size over a 2-week period. *H. ambulans* was frequently noted to be cannibalistic to the extent of attacking and sucking the body fluids of its larvae. Small psocids and collembolons placed with the mites also were attacked actively and sucked dry in a few minutes. Larger specimens were less readily attacked except when they were injured. Similarly, small specimens of the sucking louse, *Neohaematopinus neotomae* Ferris, were utilized by *H. ambulans*. There seemed to be little consistency in the mode of attack on Anoplura, but it was obvious that the procedure was not merely that of tapping the abdomen to obtain the engorged blood. In some instances the abdomen was lacerated by the mite, and the engorged gut was emptied of its blood content; in others, the cervical region was pierced and the body fluids removed. Once attacked by a single mite, the arthropod prey soon became a center of attraction for near-by mites which seized and pulled the victim about in a manner reminiscent of a flock of chickens competing for a choice morsel of food. Flea larvae and larger arthropods, such as tettigoniid nymphs, termites, and large anoplurans, were attacked only if they were injured and their body fluids exposed.

The survival and reproduction of *H. ambulans* fed only on arthropods were investigated, using a culture of 25 male and female mites held in five tubes of five mites each, as described for feeding tests on heparinized blood. As a matter of convenience, a variety of injured arthropods, or arthropod parts, was provided at 24-hour intervals as a food supply, since this permitted use of large arthropods, which, if uninjured, would not be attacked by the mites. Arthropods used for food included psocids; blattids; collembolons; stratiomyid fly larvae, pupae, and adults; flea larvae and adults; and isopods. The mites fed freely on these materials. For purposes of comparison, 25 control mites were maintained under the same conditions, except that food was withheld, and only moisture was supplied. Over a period of 1 month, the test culture produced 53 offspring, as compared with two for the control culture. Thirty mites died in the test culture, as opposed to 13 in the control, but the majority of the former were the immature offspring which became stuck in the moist arthropod food. The test cultures were terminated after producing F_2 generation offspring.

As indicated in Table 1, *H. liponyssoides* did penetrate the intact skin and feed on *Microtus* and the laboratory mouse. Adult hosts with intact skin proved unacceptable to *H. liponyssoides* under the vial technique. In successful tests, mites were allowed access to suckling mice held in a small dish at room temperature at a relative humidity of over 90 per cent. Suckling *Microtus* suffered a marked reaction to the bite; a punctate, hemorrhagic area was formed at the site of feeding, becoming surrounded by a rather extensive zone of diffuse, subcutaneous hemorrhage. In subsequent rearing studies, to be reported in detail elsewhere, adult *H. liponyssoides* have fed regularly on suckling laboratory mice, piercing the skin and producing reactions similar to that described for *Microtus*. This mite has also been collected with its mouthparts inserted in the

skin of wild suckling *Microtus*, producing the characteristic reaction. In laboratory tests, the mites appeared to be attracted to *Microtus*. Although the mites fed on liquid blood or serum, they did not feed on dried blood nor did they display predacious tendencies.

Using the vial feeding technique *H. oudemansi* fed on heparinized mouse blood but refused to feed through either the intact or abraded skin of *Neotoma*, laboratory mouse, or man, nor would it feed on dried blood. As with *H. ambulans*, the mite appeared to be repelled by close contact with a potential host.

B. utahensis was demonstrated to feed readily on heparinized or dried blood of laboratory mice and upon the abraded skin of man, but failed to feed through the intact skin of *Neotoma*, *Microtus*, laboratory rat, laboratory mouse, or man.

E. echidninus was shown to feed on heparinized or dried mouse blood and on serous exudate on the abraded skin of *Neotoma*, but it refused to penetrate the skin of various hosts, as indicated in Table 1. It was shown to be predacious on occasion. Mites maintained in the laboratory on white rats and on *Neotoma* were observed to emerge from the fur to feed on lachrymal fluids and exudate around the eyes of the hosts. Under the free-access culture technique, *E. echidninus* was associated with the development of lesions on the foot pads of suckling mice. Small abrasions were also noted on the foot pads of control mice but they were less extensive than when mites were present. No evidence was found that this mite initiated skin penetration.

H. glasgowi proved to be a rather general feeder, utilizing heparinized or dried mouse blood, as well as attacking and feeding on small anoplurans in the manner described for *H. ambulans*. It appeared to be attracted to the intact as well as abraded skin of various warm-blooded hosts, but although mites, applied by the vial technique, probed the intact skin of man and *Neotoma*, and even appeared to feed through it, no blood appeared in their bodies.

To clarify the role of *H. glasgowi* as a skin penetrator and feeder, 21 female and five male mites were cultured on suckling laboratory mice by the free-access technique. One control container identical except for the exclusion of mice, held 19 female and four male mites. A second control chamber contained a suckling mouse but no mites. Mice used were about 3 to 5 days old, free from skin lesions or umbilical scabs. *H. glasgowi* produced relatively large wounds, having a punched out appearance, on various parts of suckling mice. The tissues appeared to have been rasped or cut away until a flow of lymph was obtained. Apparently several mites fed at a single lesion, since more than two or three such wounds were rarely produced on a single mouse. Lesions of the foot pads were also encountered, but as indicated previously, these may not have been initiated by the mites. After 17 days the test-culture population had increased almost fourfold, while that of the control culture had decreased to less than one half the original number. Many of the test mites appeared to be well distended, but only one showed a dark, blood-like residue in its body. By contrast the surviving control mites appeared flattened. The control mites displayed a questing response as described for similarly-maintained *H. ambulans*. This response was absent in the test mites.

Under conditions comparable with those used in feeding tests on other mites by the vial technique, the known skin penetrator and blood feeder, *O. bacoti*, was shown to penetrate the intact skin and feed on *Neotoma*, *Microtus*, and the laboratory rat. The mite did not invariably feed on these hosts, and on the relatively thick-skinned *Neotoma* it fed only on areas of delicate skin around the genitalia. *O. bacoti* appeared attracted to the skin of the hosts, including man, and invariably fed freely on serous exudate or blood from any existing abrasion.

DISCUSSION

Of the seven species of mites tested, only two, *H. liponyssoides* and *O. bacoti*, fed readily through the intact skin of the living vertebrate host. However, *H. glasgowi* proved capable of rasping delicate skin until tissue fluids were available, and circumstantial evidence indicates that on rare occasions *H. ambulans* can penetrate the intact skin to feed on blood. When evidence of little, or no, ability to penetrate host skin is coupled with that of other food habits, the need for an obligatory, skin-penetration relationship ceases to exist. Thus, *H. ambulans* emerges as a generalized feeder, utilizing as food living and dead arthropods, dried blood, flea feces, and blood or tissue fluids of vertebrates when available. It is able to

live and reproduce on a diet composed solely of arthropods or of dried blood. *H. liponyssoides*, on the other hand, appears to be an obligatory, bloodsucking parasite, capable of piercing the intact skin of the host for blood. On the basis of food habits, a mite such as *H. liponyssoides* is far more likely to serve as a vector of parenterally-borne pathogens of vertebrates than a mite such as *H. ambulans*. *H. glasgowi* must also be considered as a suitable potential vector for parenterally-borne pathogens, for it penetrated the intact skin of a vertebrate host in addition to utilizing numerous other food sources. On the basis of evidence presented here, opportunities for parenteral transmission of microorganisms by *H. ambulans*, *B. utahensis*, and *E. echidninus* would be associated primarily with occasions when the mites feed on pre-existing lesions of the vertebrate host.

The association of rodent nests and mites with generalized food habits is favored by the varied and rich food supply available in the nests. Parasitic and free-living arthropods are common residents of the nests, and feces of vertebrate and arthropod residents are present. These food sources are supplemented by blood, both liquid and dried, as well as tissue exudates from the commonly encountered skin abrasions of wild rodents. Such a nest environment provides ample opportunities for scavenging or predacious arthropods to develop parasitic relationships with vertebrate residents. Data reported here illustrate varying degrees in the development of such a parasitic relationship. Within a single genus exist the generalized feeder and facultative parasite, *H. ambulans*, together with the obligatory parasite, *H. liponyssoides*.

SUMMARY

Feeding tests on six species of laelaptid and haemogamasid mites and one species of dermanyssid mite are reported. *Haemogamasus ambulans* (Thorell) appears to have generalized tastes, feeding on fluid or dried blood of vertebrates, flea feces, and living or dead arthropods. Reproduction and indefinite maintenance on diets of arthropods or dried blood were demonstrated. Skin penetration of vertebrate hosts was exhibited only rarely. *Echinolaelaps echidninus* (Berlese) and *Haemolaelaps glasgowi* (Ewing) also proved to be general feeders, but additionally, the latter species attacked intact, vertebrate skin, feeding on exuding tissue fluids. In limited tests, *Brevisterna utahensis* (Ewing) did not penetrate intact skin but fed quite readily on either dry or fluid blood. *Haemogamasus oudemansi* Hirst fed on free, liquid blood, but refused to penetrate skin or to feed on dry blood. *Haemogamasus liponyssoides* Ewing appears to be an obligatory blood feeder. It penetrated intact host skin and engorged on blood. It would not feed on dried blood or on small arthropods. *Ornithonyssus bacoti* (Hirst), serving as a control, pierced the host skin and fed on blood.

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A METHOD OF COMPARING GEOGRAPHICALLY DISTANT SNAIL POPULATIONS BY STUDY OF THEIR SHELL COMPOSITION[1]

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In attempts to improve the condition of laboratory-reared Oncomelanid snails it became desirable to compare the laboratory-reared snails with their field counterparts. Since *Oncomelania* is found only in the orient where it serves as the intermediate host of the blood fluke, *Schistosoma japonicum*, logistical difficulties became a factor in such comparisons. As the principal persistent part of a snail is its shell, and as this shell is composed of both an organic and inorganic portion, it seemed possible that a basis for comparison might be obtained from it. Reference to the literature revealed only the work of Vinogradova (1949) who compared the organic constituents of various Black Sea invertebrates at various times of the year. He found that the organic constituents of the animals were highest in spring and fall coinciding with the increased plankton of those seasons. Roche *et al.*, (1951) studied the organic composition of mollusk shells and found them to be composed of scleroproteins, the amino-acid composition of which varied with the species.

MATERIALS AND METHODS

The shells of *Oncomelania nosophora* from the field were obtained in quantity from specimens collected alive in Japan. These were cleaned by allowing the contents to rot and then washing and drying the shells. Cultured dead snails were sorted from the laboratory dishes as the snails were cared for, and similarly cleaned.

During initial work individual shells were measured, weighed, and placed in small "pH beakers" to which were added about 2 ml of 5% HCl. These beakers were placed in Petri dishes which, in turn, were placed in a vacuum desiccator to remove the air from the shells and to give more even decalcification. When completely decalcified, the organic shells were pressed on filter paper to remove moisture and then returned to other dry pH beakers similarly placed in Petri dishes. After being dried thoroughly under vacuum, the decalcified shells were weighed.

Two difficulties with this method were 1) Chainomatic balances weighing only to 0.1 mg were not sufficiently sensitive to weigh the extremely light organic shell with a high degree of accuracy, and 2) inclusions tended to cause gross inaccuracy. However, this method is included because it is of value when large numbers of shells are not available. If discriminatingly used, significant results may thus be obtained.

The bulk method later used confirmed the findings of the above method. Shells were measured in a 25-ml graduated cylinder, after which they were weighed and placed in a beaker with 6N HCl and left overnight. The acid was then diluted further to prevent damage to the filter paper. The material was poured down the sides of a weighed filter paper and the shells which stuck to the walls of the filter paper cone were removed and placed in distilled water. The debris was left on the filter paper and after drying was weighed, the true weight of the debris being subtracted from the total shell weight. After thorough washing and drying, the organic shells were also weighed and the percentage of organic material calculated.

The findings of Roche *et al.*, (1951) indicated the organic material of the shell to be scleroproteins. The shells were checked for nitrogen and sulfur, using the sodium fusion technique as described by McElvain (1945). The shells were refluxed in 6N HCl for 6 to 24 hours, evaporated to dryness and reconstituted with distilled water. This solution was then made alkaline to precipitate the $CaCO_3$. The resulting filtrate was concentrated and assayed qualitatively for amino acids.[2]

[1] It is recognized that some unavoidable variables may have been present in the material at hand. This necessitates the emphasis being placed upon the method rather than the results obtained.

[2] The writers are indebted to Dr. Rita Schafer of the Allan Hancock Foundation, University of Southern California, for running the large, two-dimensional chromatograms.

EXPERIMENTAL RESULTS

The results of the weighing of individual shells are best shown in Table 1. The principal objection to this method is that inclusions which influence the results are difficult to remove. Shells having noticeable amounts of such inclusions were not included in this table. The results obtained by the bulk method are summarized in Table 2.

TABLE 1

*Laboratory and field shells compared individually**

Snail No.	Number of whorls	Length	Width	Length + width	Total weight	Organic weight	Per cent organic matter
		Laboratory specimens					
1	7	43	19	62	16.3	0.2	1.24
2	5 (= 7 − 2)	38	18	58	7.5	0.1	1.33
3	7	45	19	64	19.0	0.3	1.58
4	7	48	21	69	18.7	0.1	0.54
5	5 (= 6 − 1)	30	15	45	7.5	0.2	2.67
6	5 (= 7 − 2)	43	21	64	28.4	0.2	0.71
7	7	41	18	59	11.3	0.1	0.89
8	6 (= 7 − 1)	44	19	63	15.1	0.1	0.66
9	7	41	19	60	12.9	0.1	0.78
					Total 10.20, Average 1.13%		
		Field specimens					
1	7	40	17	57	8.9	0.2	2.24
2	7	45	18	63	15.8	0.4	2.53
3	7	44	18	62	11.4	0.3	2.64
4	6 (= 7 − 1)	45	20	65	15.5	0.2	1.29
5	5	25	13	38	3.3	0.1	3.02
6	5	19	11	30	2.3	0.1	4.78
7	6 (= 7 − 1)	45	21	66	18.0	0.4	2.26
8	6	30	15	45	6.6	0.05	0.76
					Total 19.42, Average 2.43%		

* Dimensions are relative units; weight in mg.

TABLE 2

Results obtained by the bulk method of comparing shells from the laboratory and field*

ml	Weight of sample	Weight of trash	True weight	Total organic matter	Organic matter per ml	Per cent organic matter	Source
9	4,118	568	3,550	11.1	1.233	0.31	Lab.
15	6,030	116.3	5,914	35.8	2.387	0.6	Japan
15	6,421	698	5,723	33.7	2.247	0.59	Japan

* All weights in mg.

The elementary analysis gave a strong nitrogen indication, but none for sulfur. The paper chromatography showed the following amino acids of which glycine was predominant: aspartic acid, glutamic acid, serine, glycine, threonine, alanine, and arginine.

DISCUSSION

The individual-shell method of ascertaining the percentage of organic matter present, while a very convenient, and on occasion the only applicable, method has the serious disadvantage of not accounting for inclusions. While the individual shells varied considerably in percentage of organic material present, the overall average gives results closely comparable with those obtained by the dissolution method, showing that the snails in the laboratory lay down approximately one-half as much organic material in their shell as their Japanese counterparts.

The lower percentage of organic material obtained by the bulk method is due to the use of more concentrated acid (6N) for dissolution. The bulk method is considered the more accurate. The 5 per cent HCl dissolution of the inorganic material, as used for individual shells, leaves considerable inorganic residue which is effervescent in acid. By ignition, this was determined to constitute 13.8 per cent of the so-called organic component. Furthermore, the stronger acid dissolves a portion of the organic matter. This is made evident, in dissolution with 6N HCl, by the color of the dissolved material. Concentrated acid completely dissolves the organic shell in about a week. In the bulk method the inclusions are collected and weighed, a procedure which insures greater accuracy. The exact amount of organic matter is not important. Providing the same method of preparation is used for all of the compared materials, the comparison should be valid.

The indication that considerably more nitrogen and amino acids are present in the shells of the snails obtained in nature than in those raised in the laboratory suggests that nitrogenous food may be lacking in the laboratory culture dishes and may thus be a factor of critical importance in the life of these snails. The excess feeding by the snails on the purified cellulose

of the filter paper in their dishes, despite their ability to digest it (Winkler, in preparation), could well result in abnormalities due to nutritional deficiencies.

It is felt that the method of studying the composition of snail shells described here may have applications in studies of other animals possessing persistent skeletal material.

SUMMARY

1) The percentages of organic matter in shells of laboratory-raised *Oncomelania nosophora* and in those of their field counterparts were determined by both individual and bulk methods.

2) Simple chemical analyses indicated considerable nitrogen and some amino acids present in the organic material of the shells.

3) Shells of laboratory-raised snails contained only about one-half as much organic matter as those of field snails.

4) It is thought that this indicates a possible insufficiency of nitrogenous food in the laboratory culture dishes. This may be due in part to an excess eating of refined cellulose.

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THE LONGEVITY OF THE UNHATCHED MIRACIDIUM OF *SCHISTOSOMA MANSONI* IN THE TISSUES OF MICE[1]

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The longevity of the miracidium of *Schistosoma japonicum* in the tissues of the mammalian host has been studied by Vogel (1942). He isolated the smallest lobe of the liver of infected rabbits by tying the branches of the portal vein. Examination of the isolated lobe of experimental animals, at various intervals of time after tying the veins, led to the conclusion that all of the eggs were dead after 22 days. He also assumed that the unhatched, fully-formed miracidium, could survive in the tissues for only 11 or 12 days, since he thought that 9 to 19 days were required for the miracidium to develop to maturity after oviposition.

Looss (1908) believed that eggs of the African schistosomes, *Schistosoma haematobium* and *Schistosoma mansoni*, would survive in the tissues for years, but Brumpt (1930) thought that the life span was only a matter of several weeks or months. Recently, Kagan and Lee (1952) reported that the eggs of *Schistosomatium douthitti* survived in the mouse liver for 3 to 4 weeks, and Gonnert (1955) reported the same period, or less, for the eggs of *S. mansoni*.

Except for the report by Vogel (1942), none of the papers of recent workers has been sufficiently critical to provide a definitive answer on this subject. The present paper gives the results obtained from experiments devised to make a precise determination of the longevity of the miracidium within the egg of *S. mansoni*, as found in the tissues of infected mice.

MATERIALS AND METHODS

Preliminary experiments with rabbits, following the method of Vogel (1942) were unsuccessful because the rabbit is not a good host for infection with *S. mansoni*. Although the parasite did develop, it did so in an unpredictable manner, and egg production was very irregular.

Kagan and Lee (1952) reported that tri-(n-dodecyl)-mercapto-s-antimonious acid killed the adult worms quickly, but did not affect the eggs in the tissues. In the present experiments this drug[2] was injected intraperitoneally into mice that had been infected 2 months previously. The drug was suspended in sesame oil and 225 mg per kg of body weight were administered on 2 consecutive days. Animals were examined during the period from the first to the 35th day after the second injection. An average of three animals were sacrificed on selected posttreatment days (Figure 1), a total of 79 mice being employed in all the work.

The worms recovered were studied and their location in the body recorded. Pieces of liver and intestine were pressed between glass slides, and differential counts of the living and dead eggs were carried out. The starting date for studying longevity of the eggs in the tissues was the day following the second of the two consecutive injections of the drug. When living eggs were abundant, the counts did not exceed 100 eggs. As their number decreased, the search was extended to include several hundred of all types. In the terminal stages of the study, every effort was made to examine as much tissue as possible.

One or more of the following criteria for determining death of the miracidium were employed: the presence of deformities, vacuolization, the absence of active flame cells, a definite outline of the miracidium within the egg, any sign of movement, and a loss of turgidity by the egg. These characteristics had been used previously in the study of the viability of the egg in the external environment (Maldonado *et al.*, 1949).

Hatching tests were carried out by placing normal-looking eggs in water, exposing them to bright light, and examining them at intervals with a dissecting microscope. During the posttreatment period, feces were collected and ex-

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[2] The author wishes to thank the Abbott Research Laboratories and Dr. Irving Kagan for their help in providing materials for this experiment.

amined for eggs by concentrating with formalin-Triton-ether (Maldonado *et al.*, 1954).

RESULTS AND OBSERVATIONS

Effect of drug on the worms. The ovary showed the alterations described by Bang and Hairston (1946), which indicated that egg production had been suppressed as early as the third day following treatment. Some of the worms migrated toward the liver, while others in the portal trunk became paralyzed, and appeared to be dead. This has permitted selection of the first posttreatment day as the starting date to determine the life span of the egg.

By 4 days, migration into the liver was more noticeable, but only after 8 days had elapsed did all the worms move into this organ. After this period the female worms decreased in size and acquired a juvenile appearance due to regression of the vitellaria and cessation of feeding on blood. After 11 days normal worms were only occasionally encountered in the liver. Finally, the parasites became enveloped in a tissue reaction that could sometimes be seen as white patches on the surface of the liver. On rare occasions, some worms would die in the mesenteric veins in much the same manner.

Effect of drug on the eggs. Dead and dying eggs are encountered, after their deposition, in the tissues of the mammalian host after infection for varying lengths of time. In seven untreated animals infected for 2 months, the percentage of dead eggs varied between 10.0 and 29.8 in the liver, with an average of 21.4. Similar observations were made of eggs deposited in the intestinal wall. The percentage of dead eggs increased with the age of the infection (Gonnert, 1955).

Figure 1 illustrates the proportions of live eggs from the first posttreatment day in the liver and intestine. An unexplained drop in their percentage was observed on the third day in both organs among the five animals studied, after which the rates returned rapidly to the original levels. After 6 to 12 days, the proportion of live eggs gradually decreased, until by 33 days no more of them were encountered.

Hatching of miracidia from eggs in intestinal tissue occurred as late as the 26th day, but the

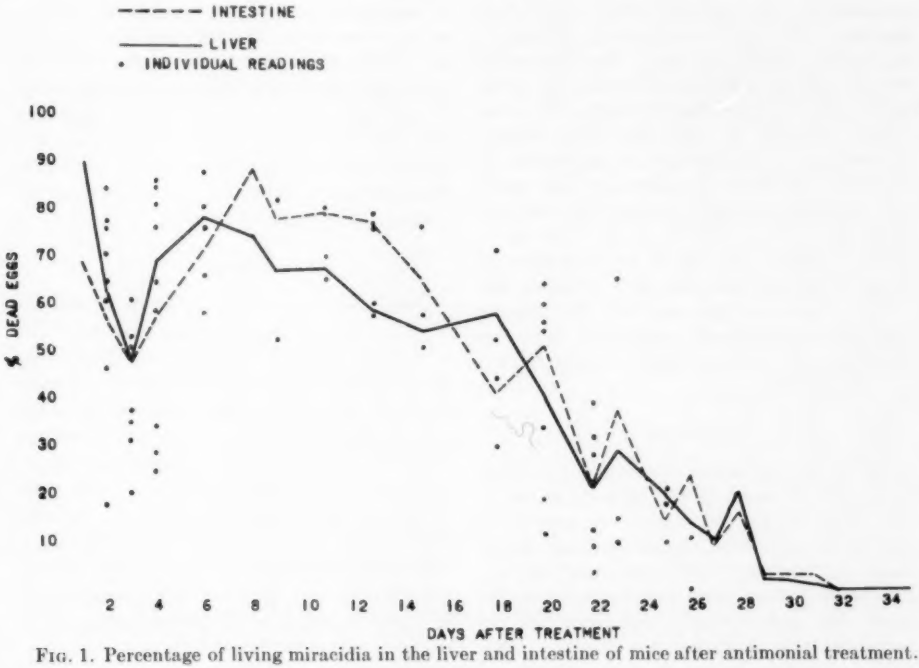

FIG. 1. Percentage of living miracidia in the liver and intestine of mice after antimonial treatment.

larvae swam slowly and were short-lived. Similar experiments with eggs from the liver were successful at 23 days, but after that period all those observed were encased in pseudotubercles, and thus were physically unable to hatch.

No eggs could be revealed in the feces, by the techniques employed, 6 days after treatment. Beyond this period the intestinal wall appeared increasingly free of eggs, and those that could be spotted were located in the *tunica muscularis* and the *serosa*, many of them in pseudotubercles.

Mode of death of the egg. The deterioration and death of the schistosome eggs in the tissues ensues in practically the same manner as of eggs recovered from feces and held in the laboratory (Maldonado *et al.*, 1949). The normal, living egg has the same appearance, whether in tissue or in feces. In addition to the perfectly outlined miracidium and the turgidity, the amber coloration and one or two crystalline droplets of undetermined nature within the egg are characteristics of the living state. These droplets, depending on the orientation of the egg, may appear as transverse internal bands. This feature and the color of the egg are gradually lost as a greyish, opaque granulation progressively accumulates within the shell, starting at the poles and partially hiding the miracidium as the egg ages. The miracidium remains patent in the middle of the egg where the granules are less dense. Finally, the miracidium decreases in size and often assumes bizarre shapes, even though the egg retains its turgidity. The flame cells gradually slow down, but sometimes continue beating even after the larva becomes vacuolated. A point of uncertainty regarding viability of the miracidium is reached approximately 25 days following deposition. Once the eggs are dead they become discolored, progressively smaller as they lose turgidity, and the miracidium is reduced to a granular residue.

DISCUSSION

One of the difficulties encountered in determining the life span of the miracidium within the egg of *S. mansoni* depends on whether it undergoes development outside the female worm. Faust and Hoffman (1934) state that from the time of oviposition, until eggs are found in the stools, they mature rapidly. Gönnert (1955) reports that the embryonic development of the egg outside the worm lasts about a week. Our observations indicate that a mature miracidium of *S. mansoni* is present at oviposition, and that there is no necessity for extrauterine development. If any eggs were laid in an immature condition, the curve (Fig. 1) for the second and third posttreatment day would be directed upward, instead of downward, inasmuch as the percentage of living eggs would increase during those days with their coming into maturity. If any immaturity exists in the newly-laid egg, it could be physiological instead of morphological (Maldonado and Acosta-Matienzo, 1948). What have heretofore been called the immature stages of *S. mansoni* eggs are, to all appearances, dying eggs which show discoloration, and a miracidium masked by the accumulating excretory deposits. Such eggs might have been normal, or abnormal ones in which the miracidia never reached maturity. Some evidence in favor of the latter hypothesis has been gathered in the present experiment.

A longevity of 32 days for the egg of *S. mansoni* exceeded expectations. Although alive, many of the eggs cannot be considered normal during their last days of life, since their chances of survival in an adequate medium are already greatly reduced by the 26th day after oviposition. From the point of view of the possibility of a miracidium hatching with enough energy to accomplish parasitization of the molluscan host, the effective life span must be considerably shorter. The only importance of the protracted life of the larva within the host would lie in the antigenic stimulation of the host.

SUMMARY

Destruction in mice of the adult *S. mansoni* after 2 months of infection, by treatment with an antimonial drug, has permitted establishing 32 days as the longevity of the egg in the tissues. Evidence indicates complete development of the miracidium at oviposition. With the passage of time, there is a gradual accumulation of excretory deposits within the egg, the amber color of the egg fades, and the miracidium becomes smaller and develops deformities. The flame cells of the miracidium continue to show activity until the death of the larva. These degenerative changes are similar to changes occurring in the egg recovered in the feces of the host.

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HOST-PARASITE RELATIONSHIPS IN ECHINOCOCCOSIS

I. Observations on the Permeability of the Hydatid Cyst Wall[1]

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While knowledge of the permeability of the hydatid cyst wall (HCW) appears to be basic to studies on hydatid metabolism, therapeutics and immunology, little attention in the past has been directed to this question. Dévé (1926, 1936) recognized the importance of permeability studies, but felt that they must wait upon successful in vitro cultivation of the hydatid cyst. Much earlier, Schilling (1904) had placed cysts in distilled water for 48 hours and noted at the end of that period, that the NaCl concentration and freezing point of the hydatid cyst fluid (HCF) and the surrounding medium were the same. Codounis and Polydoridès (1936) compared the concentrations of several ions and nonelectrolytes in blood sera and HCF and found that they were, for the most part, in close aggreement. Mazzacco (1923), who also examined the chemical composition of HCF, likened it to cerebrospinal fluid and surmised that the cyst wall must be permeable to "*substances tres diffusibles.*" Wernicke and Savino (1923) studied certain physical properties of HCF and noted that, for data associated with electrolyte concentration, HCF and blood serum values were similar, but data dependent upon protein concentration were not. These authors suggested that the HCW does not differ in behavior from other animal membranes.

Similarly, Dew (1928) assumed the selective permeability of the HCW but offered no supporting evidence. On immunologic grounds, Lemaire (1926) conjectured that active antigenic substances must diffuse through the HCW into the tissues of the host. The present paper is the first of a series on biological aspects of the helminth-host relationship in echinococcosis, and on the physiology of *Echinococcus granulosus*.

MATERIALS AND METHODS

The results presented here were obtained by measuring changes in weight of small hydatid daughter cysts (HDC) of *E. granulosus* immersed in solutions of varied tonicity and composition. The HDC were obtained from bovine lungs and livers delivered to the laboratory from the Beirut Municipal Abbatoir within 2 hours after slaughter of the host animals. Small HDC were removed to beakers of HCF and were used immediately or occasionally stored at 8°C for not more than 24 hours. Most of the HDC used had an initial weight of 100 to 300 mg. The cysts were lifted from the solution on curved, stainless-steel forceps, gently blotted on filter paper and weighed to the nearest 0.1 mg on a precision torsion balance (Roller-Smith). Less than 0.5 minute elapsed between removal of the cyst from the fluid and its replacement. Beakers containing the solutions being examined were partially immersed in a constant-temperature serological bath at 37°C. The ratio of solution volume to cyst volume was approximately 100:1. Cysts were weighed during a 2.5- to 3-hour period. Following the example of Wardle (1937) with *Monezia expansa*, measurements were reduced to weight per original mg.

Isosmotic solutions of electrolytes were calculated from freezing-point determinations with the Beckman apparatus.

Chemicals were of reagent grade; biochemicals were produced by Nutritional Biochemical Corporation, Cleveland, Ohio.

RESULTS

Initial experiments showed that the variation in osmotic behavior between HDC obtained from different organs or from different host cattle was no greater than that between HDC from the same mother cyst. Furthermore, no differences were noted between fresh HDC and those held in HCF at 8°C for 24 hours[2] nor between cysts of different sizes (within the weight range selected).

[1] Aided by grants from the National Institute of Allergy and Infectious Diseases, U. S. P. H. S. (E-1769), and Pfizer International.

[2] Clunies-Ross (1929) found that storage at 4 to 8°C for 8 days did not impair the viability of *E. granulosus* scolices; Agosin *et al.*, (1957) observed no decrease in oxygen consumption of scolices following 24-hour storage at 5°C.

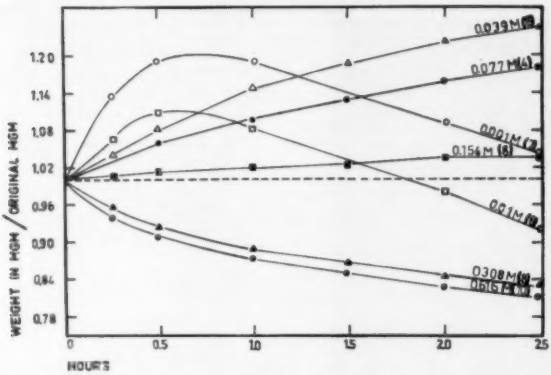

FIG. 1. Change in weight of hydatid cysts suspended in NaCl solutions of different molarities.

Water and electrolytes. As shown in Figure 1, water passed rapidly through the HCW when cysts were immersed in hypertonic and hypotonic NaCl solutions at 37°C. Passage of water was less rapid at 25°C. In hypotonic NaCl solutions of 0.25 isotonic concentration or less, the selective permeability of most cyst walls broke down following an initial period of water imbibition at the expected rate. This is shown in Figure 2 for individual HDC suspended in glass-redistilled water. Similar effects were obtained in tap water, 0.001 M NaCl containing 0.001 M KCl, sheep serum diluted 1:4 or more, and diluted HCF. The cause of this sudden permeability change is being studied further. Possibly some HCW substance essential to permeability control is dialyzed from the wall in hypotonic solutions.

In sheep serum and in human serum, HDC lost weight slowly, but gained slightly in NaCl solution isosmotic with rat serum. The behavior of HDC immersed in various salt solutions[3] isosmotic with rat serum is shown in Table 1. Some differential permeability to different ion mixtures was apparent.

In subsequent experiments, unless otherwise indicated, the suspending solutions contained 0.154 M NaCl plus the particular substance being examined. (The mean Na content of bovine cyst fluids is 124.9 mE/liter, unpublished data). From determinations of their freezing-point depressions, hypertonic solutions of NaCl, KCl and $CaCl_2$ were prepared which were isosmotic to one another and to 0.7276 molal nonelectrolyte. Although weight changes after 3 hours of immersion in these solutions at 37°C showed (Table 2) slight apparent differences in the rate of permeation of the laminated membrane, the differences were not statistically significant. A marked qualitative change, however, was noted in the HDC suspended in KCl. In this medium the inner germinal membrane progressively withdrew from the laminated membrane (Figure 3). Differential permeability of the two membranes was suggested. Similar withdrawal was observed during a 3-hour period in HDC incubated in 0.616 M KCl alone, 0.154 M NaCl plus 0.462 M KH_2PO_4, 0.154 M NaCl plus 0.4 M K_2SO_4, but not in 0.154 M KCl. The membrane separation was less marked in the KH_2PO_4. This apparent alteration in the permeability of the germinal membrane appeared, therefore, to result from an effect of K^+ present in hypertonic solutions. The germinal membrane was shown to be nonadherent to the laminated membrane and to constitute a delicate yet distinct membrane.

Preliminary experiments to compare the rates of permeation of different anions of sodium indicated that the chloride, fluoride, bicarbonate and acetate were all within the same general range. In other preliminary experiments, $MgCl_2$ and $MnCl_2$ fell within approximately the same range as NaCl, KCl and $CaCl_2$.

Nonelectrolytes. Experiments similar to the above were run with isosmotic solutions (0.7276 molal) of urea, glycerol, mannitol, xylose, arabinose, fructose, glucose, galactose, maltose and sucrose. Table 3 shows the relative permeability of the laminated membrane to these substances. (Values in Tables 2 and 3 are comparable). In general they arranged themselves in

[3] Prepared according to Cohen, in Umbreit *et al.*, (1949: p. 119).

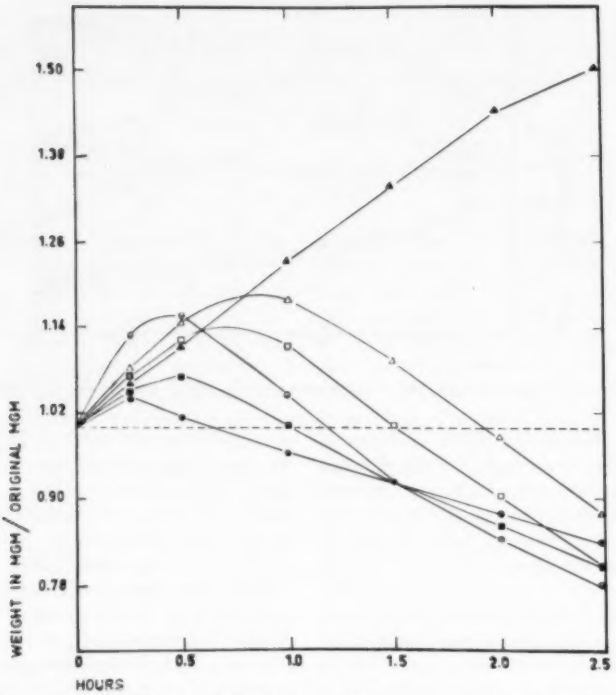

FIG. 2. Change in weight of individual hydatid cysts suspended in distilled water.

order of increasing molecular weight. While urea permeated the laminated membrane most rapidly the germinal membrane withdrew markedly in its presence, suggesting a differential permeability to this compound. It may be pointed out here, as of possible significance, that the urea content of HCF is generally higher than that of the host's blood (Codounis and Polydoridès, 1936). The accumulation within the HCF of substances which slowly permeate or do not permeate the germinal membrane very likely relates to cyst growth. Some differential permeability was also apparent with arabinose and xylose. Figure 4 shows the relative permeability of the laminated membrane to glycerol, sucrose and Mercurochrome, the last a presumably nonpermeating substance of high molecular weight (Sollman, 1950). As the freezing-point depression of Mercurochrome solutions has not been determined, these curves are only approximate.

Permeability regulation. Wilbrandt and Laszt (1933) found that iodoacetate prevents the rapid absorption of glucose by the cells of the intestinal mucosa. More recently Agosin *et al.*, (1957) have shown that 0.001 M iodoacetate inhibits the respiration of *E. granulosus* scolices by 76 per cent. Accordingly, the effect of iodoacetate upon the permeability of the HCW to glucose was examined. HDC were suspended in 0.7276 molal glucose-0.154 M NaCl and in glucose-saline containing 0.01 M iodoacetate. No difference in osmotic behavior between the two groups during a 3-hour period was observed. This indicated that glucose transport through the laminated membrane is probably not mediated by enzymes inhibited by iodoacetate.

The observed differential effect of potassium suggested that possible iodoacetate effects upon the permeability of either membrane could be noted in this case. Cysts were suspended for 3 hours at 37°C in 0.384 molal KCl-0.154 M NaCl and in KCl-NaCl containing 0.01 M iodoacetate. The effect of iodoacetate was markedly antagonistic to that of potassium; little, if any, separation of the germinal and laminated membranes occurred. Iodoacetate did not, however, affect the

TABLE 1

Change in weight of hydatid cysts suspended at 37°C for 2.5 hours in solutions isosmotic with blood serum

Constituents of solution	Proportions (*parts*)	Mean weight in mg per original mg ± S.D.
0.154 M NaCl	100	1.015 ± 0.0797 (8)†
0.154 M NaCl 0.154 M KCl	100 4	1.014 ± 0.0515 (10)
0.154 M NaCl 0.11 M $CaCl_2$	100 3	1.011 ± 0.0424 (6)
0.154 M NaCl 0.154 M KCl 0.11 M $CaCl_2$	100 4 3	0.955 ± 0.0215 (4)
0.154 M NaCl 0.154 M KCl 0.11 M $CaCl_2$ 0.154 M KH_2PO_4 0.154 M $MgSO_4$	100 4 3 1 1*	0.941 ± 0.0123 (12)
0.154 M KCl	100	0.981 ± 0.0354 (8)
0.154 M KH_2PO_4	100	1.005 ± 0.0364 (4)
0.154 M $MgSO_4$	100	1.125 ± 0.0924 (8)

* Krebs-Ringer's solution

† Figures in parentheses in tables and figures refer to the number of HDC.

TABLE 2

Change in weight of hydatid cysts suspended at 37°C for 3 hours in hypertonic electrolyte solutions (0.154 M NaCl plus an electrolyte concentration isosmotic with 0.7276 molal nonelectrolyte)

Constituents of solution	0.400 Molal NaCl 0.154 M NaCl	0.384 Molal KCl 0.154 M NaCl	0.280 Molal $CaCl_2$ 0.154 M NaCl
Mean weight in mg per original mg ± S.D.	0.790 ± 0.066 (10)	0.812 ± 0.097 (44)	0.834 ± 0.045 (10)

permeability of the laminated layer. Iodoacetate exerts its metabolic effect through inhibition of sulfhydryl-containing enzymes (Massart, 1950). Cholinesterase and choline acetylase are among these.

As Cicardo and Moglia (1940) had demonstrated that potassium is liberated from muscle perfused with acetylcholine and Holland and Greig (1950) had shown an effect of acetylcholine upon the permeability to potassium of dog and cat erythrocytes, it was decided to study the

FIG. 3. Withdrawal of the germinal membrane from the laminated membrane as occurs in the presence of potassium ion, cyanide ion and urea.

possible role of acetylcholine-cholinesterase in the hydatid cyst wall. Changes in the germinal membrane were recorded each 30 minutes for 3 hours by reference to an arbitrary scale (Figure 5). Alterations in the permeability of the laminated membrane were followed by weighing the HDC as before. The osmotic behavior of HDC was compared in KCl-NaCl alone, KCl-NaCl with iodoacetate, and KCl-NaCl with 0.01 M acetylcholine chloride. Acetylcholine also prevented germinal membrane withdrawal in the presence of potassium (but not to the extent of iodoacetate). It had no effect upon the laminated membrane. Similar experiments compared the effects of KCl-NaCl alone, KCl-NaCl with acetylcholine, KCl-NaCl with 0.01 M physostigmine salicylate and KCl-NaCl with acetylcholine plus physostigmine. Physostigmine effects were identical with those of acetylcholine. Physostigmine plus acetylcholine had no greater effect than did either alone.

The above evidence suggested that the permeability of the germinal membrane, but not the laminated membrane, was influenced by an acetylcholine-cholinesterase system. Further study is being directed to this question.

Attention was turned to other possible mechanisms of permeability control. Agosin *et al.*, (1957) had shown that the respiration of hydatid scolices was reduced not only by sulfhydryl inhibitors but also by 0.0005 M KCN (47%) and 0.01 M NaF (18%). The effects of KCN (0.001 M) and NaF (0.01 M) upon HCW permeability

TABLE 3

Change in weight of hydatid cysts suspended for 3 hours in hypertonic solutions of nonelectrolytes (0.154 M NaCl plus 0.7276 molal nonelectrolyte)

Substance	Molecular weight	Mean weight in mg per original mg ± S.D.
Urea	60.06	0.875 ± 0.073 (16)
Glycerol	92.09	0.803 ± 0.059 (10)
Arabinose	150.13	0.786 ± 0.045 (9)
Xylose	150.13	0.767 ± 0.048 (10)
Fructose	180.16	0.761 ± 0.072 (10)
Mannitol	182.17	0.733 ± 0.048 (8)
Glucose	180.16	0.690 ± 0.043 (15)
Galactose	180.16	0.681 ± 0.090 (14)
Sucrose	342.30	0.630 ± 0.031 (13)
Maltose	360.31	0.629 ± 0.089 (10)

were observed. In KCl-NaCl, sodium fluoride had no effect upon either membrane, but KCN very definitely hastened and accentuated the differential effect produced by K^+. Cysts were next suspended in 0.7276 molal glucose-0.154 M NaCl with and without 0.001 M NaCN. Cyanide alone caused a withdrawal of the germinal membrane, similar to that produced by potassium. The same results were obtained when galactose was substituted for glucose. While no explanation for cyanide action is immediately apparent, Massart (1950) states that cyanide, British anti-Lewisite and glutathione each relieve the inhibition of sulfhydryl inhibitors. This point is being studied further.

According to Flössner (1925), Čmelik (1952) and Čmelik and Briski (1953), the hydatid laminated membrane consists in part of a glucosamine-containing polysaccharide. Schmiedeberg (cited by Flössner, 1924) had previously characterized the membrane as chitinlike. It seemed possible, therefore, that hyaluronidase might increase its permeability. Accordingly, HDC were suspended for 3 hours at 37°C in 0.7276 molal solutions of urea, glucose, mannitol and sucrose (in 0.154 M NaCl) with and without 100 USP units of hyaluronidase per ml. Under these conditions, the enzyme did not alter the permeability of the laminated membrane to urea or sucrose. In hyaluronidase plus glucose, however, the mean cyst weight per original mg was 0.759 ± 0.028 (12) as compared to 0.690 ± 0.043 (15) in glucose-saline alone; in mannitol it was 0.764 ± 0.069 (7) with hyaluronidase and 0.733 ± 0.048 (8) without. Only the difference observed using glucose is statistically significant. It appears that hyaluronidase may have had a slight effect upon the laminated membrane but that for urea, a small molecule which penetrates the unaltered membrane rapidly, and sucrose, a large molecule which penetrates slowly, an effect of this magnitude was of no moment. Less equivocal results must probably depend upon chemical measurements plus the demonstration of hyaluronic acid in the laminated membrane.

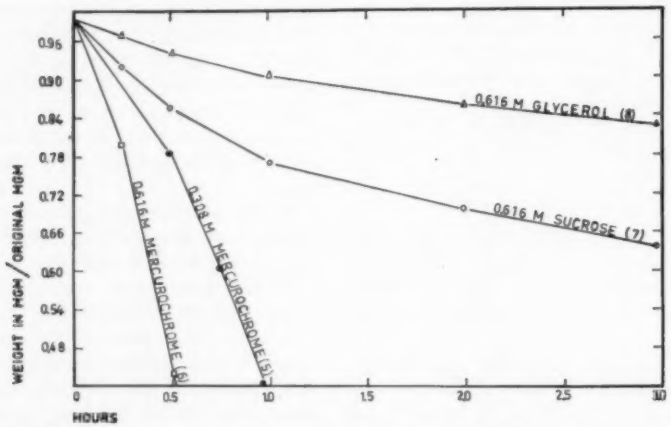

FIG. 4. Change in weight of hydatid cysts suspended in solutions of glycerol, sucrose and Mercurochrome.

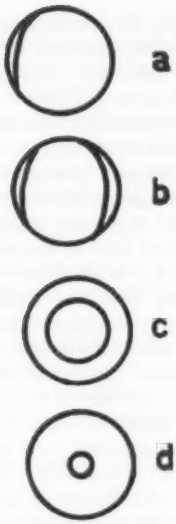

FIG. 5. Scale for determining the approximate degree of withdrawal of the germinal membrane from the laminated membrane.

DISCUSSION

The permeability of natural membranes appears to be related to the concentration, particle size, polarity, degree of ionization and oil-water-partition coefficient of the solute; chemical and morphological structure of the membrane; presence of ionic and molecular antagonists; temperature; pH; bioelectric potential; enzymatic mechanisms for active transport and a variety of other factors. Because such structurally different membranes as skin, capillary walls and those of plant cells, echinoderm ova and erythrocytes have been most studied, it is almost impossible to generalize on this subject. Contradictory results are frequently seen in the literature. It does seem quite clear, however, that results obtained with one tissue are not necessarily applicable to others. Even erythrocytes of closely related mammalian species show striking differences in their permeabilities (Davson and Danielli, 1943).

In this work several of the classical approaches to permeability study were attempted but most were discarded for various reasons as inappropriate. The passage of water and dissolved substances in and out of the intact cyst, as measured chemically and by changes in cyst weight, seemed the most suitable approach. The work to date is based upon weight changes only. It would appear that the HCW is not analogous to any of the well-studied membranes. Its two distinct membranes differ from one another in both structure and physiology. With respect to permeability they must therefore be considered independently.

These initial observations confirm, in general, the assumptions of previous workers in that small molecules and certain physiologically important ions seem to permeate both membranes readily. However, it is apparent that cyst-membrane permeability is not this simple, as has been demonstrated in the case of potassium and urea. It appears that mechanisms of active transport may be involved in the case of the germinal membrane.

In many of the studies on membranes of diverse types potassium has assumed an unusual importance. Several workers (see Brooks, 1947) have demonstrated a relationship between potassium permeability and glucose phosphorylation in microorganisms. In the case of erythrocyte permeability the role of potassium is an important one (Davson and Danielli, 1943; Davson, 1940). The presumed relationship of potassium to nervous-impulse transmission also involves questions of permeability. Potassium effects, in addition, have been observed in muscle, invertebrate cells and plant cells and tissues. Therefore, a potassium effect upon germinal-membrane permeability would not be surprising. Possible specific action in this instance is a subject for further study.

Another substance which has been demonstrated to have some relation to the permeability of a variety of natural membranes is acetylcholine (Augustinsson, 1952, 1950, 1948; Martin, 1951; Nachmansohn and Wilson, 1951). In the cases of erythrocyte and muscle permeabilities, a relationship between potassium and acetylcholine has been demonstrated (Cicardo and Moglia, 1940; Holland and Greig, 1950). In addition, K^+ is essential for the optimum activity of choline acetylase (Nachmansohn and Wilson, 1951) and, according to Everett (1946), potassium inhibits cholinesterase. Bueding (1952) has demonstrated acetylcholinesterase activity in *Schistosoma mansoni*, *Ascaris lumbricoides* and *Litomosoides carinii* and has suggested that its presence may relate to motor activity in these species.

Whether the suspected cholinesterase of the hydatid germinal membrane is the specific or nonspecific type remains to be shown, but in this instance a definite function is suggested.

In addition to throwing light upon the physiology of larval cestodes in general, studies on HCW permeability are closely related to three important aspects of this particular parasitism, namely the questions of cyst growth, chemotherapy and immunology. Our observations to date have caused us to advance several hypotheses about membrane formation and function: (1) the germinal membrane is chiefly responsible for permeability control and osmoregulation; (2) it is impermeable to certain products of its own metabolism; (3) the accumulation of nonpermeating substances results in the maintenance of an osmotic differential with respect to host fluids and the continual imbibition of host water; and (4) this explains cyst growth. With respect to the laminated membrane, we would suggest that (1) it forms as a result of a precipitin reaction between metabolic products of the germinal membrane or scolices as antigens and antibodies produced by the host; (2) that it is nonliving and (3) so far as cyst-wall permeability is concerned, acts primarily as a filter which is able to retain large molecules.

Concerning the first hypotheses, the initial results with acetylcholine and inhibitors are suggestive of a functional enzymatic mechanism for permeability control in the germinal membrane. Further evidence is provided by the observation that a small molecule such as urea, which readily penetrates a variety of natural membranes, apparently penetrates the germinal membrane at a slow rate. Were all of the metabolic endproducts of germinal-membrane metabolism able to pass freely through the membrane, the larval hydatid parasite would no doubt grow as a spongy matrix rather than as a turgid, fluid-filled sac. (The essential differences between *E. granulosus* and *E. multilocularis* might conceivably involve just such differences in physiology).

The second hypotheses concern the laminated membrane. It is quite obvious that were it not for the skeletal support lent by the laminated membrane, the delicate germinal membrane sac would not develop to a very great size without rupturing. Present evidence would not contradict the view that the laminated membrane is a nonliving structure. It is acellular and consists of concentric layers of a material with no particular structural characteristics. Its chemical composition is partially known (Flössner, 1925; Čmelick, 1952, Čmelik and Briski, 1953). In a single experiment (unpublished data) we observed that laminated-membrane homogenates consumed little, if any, oxygen, while similar preparations of the germinal membrane respired at a rapid rate. The current view of laminated-membrane formation is that it is laid down by the germinal-membrane cells (Belding, 1952). One would wonder, then, why the brood-capsule membrane, an apparently analogous structure, does not lay down a laminated membrane also. If, on the other hand, one presumes that the laminated membrane is produced only when metabolic products of the germinal cells are precipitated in the presence of antibody, the question of brood-capsule membranes is of no moment (since host protein is presumably excluded from the fluid of intact cysts). The question of daughter-cyst formation would be answered by assuming that daughter cysts arise only within injured mother cysts. In working with cattle material, in which daughter-cyst formation is frequent, it has been our observation that this is usually quite obviously the case. We have observed also that when hydatid sand is injected intraperitoneally into mice, definite laminae are laid down about individual scolices even while their morphology as scolices is still apparent (Schwabe and Schinazi, unpublished data). We are suggesting that the type of antigen-antibody precipitin reaction observed in vitro by Sarles (1938) with nematodes and by Silverman (1956) with taeniid hexacanth embryos, occurs in vivo in the case of echinococcosis. Thorson's (1953) observation that solutions containing the excretions-secretions of *Nippostrongylus muris* larvae form gummy precipitates with antibody-containing serum in vitro is also of interest in this connection, since a precipitation test employing hydatid fluid and host serum has had some diagnostic use in echinococcosis (Bryce *et al.*, 1924).

In addition to these important questions of cyst growth, HCW permeability is no doubt closely involved in the immunologic relationships between parasite and host. If it is assumed that antibody production is continuous, one may imagine that, following initial stimulation by the metabolizing naked oncosphere, stimulus occurs

through diffusion of metabolic products of the cyst wall or substances from the HCF into the body fluids of the host. Much recent work on the chemotherapy of microbial disease has also centered upon problems of permeability. Knowledge of laminated- and germinal-membrane permeabilities would assuredly be necessary in any rational approach to hydatid therapeutics.

CONCLUSIONS

1. The germinal and laminated membranes of the hydatid-cyst wall are readily permeable to water.

2. The permeability of the laminated membrane to nonelectrolytes increases in order of decreasing molecular weight. $CaCl_2$, KCl and NaCl permeate the laminated membrane at approximately the same rate.

3. The selective permeability of the hydatid-cyst wall breaks down rapidly when cysts are suspended in hypotonic solutions.

4. Potassium ion and cyanide ion, when present in hypertonic media, cause a pronounced withdrawal of the germinal membrane from the laminated membrane suggesting an effect upon permeability.

5. Urea penetrates the laminated membrane readily but, since the germinal membrane withdraws markedly in its presence, differential permeability is suggested.

6. Acetylcholine, physostigmine and iodoacetate are each antagonistic to potassium in that they prevent the withdrawal of the germinal membrane. It is suggested that a cholinesterase system may function in the osmotic regulation of the hydatid cyst.

7. Acetylcholine, physostigmine, iodoacetate, cyanide and fluoride do not affect the permeability of the laminated membrane.

8. Results obtained with hyaluronidase are equivocal. While it appears to increase the permeability of the laminated membrane to glucose, and possibly to mannitol, it has no effect upon urea or sucrose permeation.

ACKNOWLEDGMENTS

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HOST-PARASITE RELATIONSHIPS IN ECHINOCOCCOSIS

II. Age Resistance to Secondary Echinococcosis in the White Mouse[1]

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Development of hydatid cysts in the white mouse following the intraperitoneal injection of scolices of *Echinococcus granulosus* has been observed by Dévé (1933), de Waele and de Cooman (1938) and Coutelen *et al.*, (1939). This infection, roughly analogous to that following hydatid cyst rupture in the naturally infected host, has been termed secondary echinococcosis. The present paper demonstrates age resistance to secondary echinococcosis in the white mouse and describes the in vivo development of hydatid cysts from scolices.

MATERIALS AND METHODS

Thirty-two white mice representing seven litters of different ages (Table 1) were each inoculated intraperitoneally with 0.25 ml of a uniform suspension of scolices in physiological saline and with 300 units of crystalline penicillin G. Five mice of an eighth control litter were each given an equivalent number of scolices *per orum*. The mice were of a strain which was obtained from the U. S. Naval Medical Research Unit no. 3, Cairo, Egypt, in 1952. The scolices had been collected aseptically from a 1.8-kg hydatid cyst of the human brain and washed several times in sterile saline.

Mice from different age groups were killed with ether periodically. One older mouse and one younger mouse were examined each time (Table 2). Their peritoneal cavities were washed with 1 or 2 ml of physiological saline and the washings and peritoneal contents carefully examined for the presence of scolices or developing cysts. Some masses of reactive host tissue containing scolices or developing cysts were teased apart in saline and examined microscopically while others were sectioned at 10 μ and stained with hematoxylin and eosin.

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RESULTS

On the third day postinfection, the peritoneal washings of the younger mouse contained numerous healthy, invaginated scolices and comparatively few leukocytes (Figure 1). An occasional dead scolex was surrounded by a mass of white cells. In contrast, the peritoneal washings of the older mouse were milky white and contained very large numbers of leukocytes, chiefly lymphocytes and heterophils. Scolices were numerous, but were, without exception, completely surrounded by clumps of leukocytes. Most scolices were dead and degenerated (Figures 3 and 4). By the sixth day the peritoneal cavities of both mice contained numerous white, glistening masses which, in the younger mouse, were from 1 to 2 mm in diameter and, in the older mouse, up to 1 cm. Some of these were free within the peritoneal cavity but most were closely applied to various organs, chiefly to the diaphragmatic surface of the liver. When teased apart, they consisted, in the younger mouse, of large numbers of living scolices embedded in a matrix of fibroblasts and heterophils (Figure 2) and, in the older mouse, of a similar matrix containing numerous scolices, almost all of which were dead (Figure 12). In addition, the peritoneal washings of the younger mouse still contained many free scolices, approximately 75 per cent of which were normal in appearance and motile; the washings from the older mouse contained only leukocytes and masses of dead scolices embedded in host cells.

On the tenth day, the washings of both mice were devoid of free scolices, but again leukocytes were far more numerous in the older mouse than in the younger. Masses of host cells and scolices up to 1 cm in diameter were present in both mice, most being attached to the diaphragmatic surface of the liver, the spleno-gastric ligament and the mesenteries. Most of these scolices in the younger mouse were invaginated and otherwise

TABLE 1

Mice inoculated intraperitoneally with hydatid sand

Litter	Mice	Age at inoculation
I	4	89 days
II	6	81 days
III	3	71 days
IV*	5	59 days
V	5	48 days
VI	5	34 days
VII	5	22 days
VIII†	4	15 days

* Mice of control litter IV were given scolices orally.

† Unweaned at time of inoculation.

TABLE 2

Necropsy schedule for inoculated mice

Days postinfection	Mouse number*
3	IIA, VIA
6	IIB, VIB
10	IIC, VIC, VIIIA (died)
11	VIIIB (died), VIIIC (died), VIIID (died)
17	IID, VID
24	IIE, VIE
29	IA (died)
33	IIIA, VA
38	IIIB, VB
52	IIIC, VC
73	IIF, VD
94	IB, VIIA
105	VE
115	IC, VIIB
136	ID, VIIC, VIID, VIIE

* Roman numeral indicates the litter number; the letter indicates the number of the mouse within the litter.

normal in appearance. Of those in the older mouse, only a few were viable (actively motile or with functional flame cells), but even most of these had evaginated (Figure 7) and some showed the "bubble" formation characteristic of scolices maintained under certain in vitro conditions (Schwabe, unpublished observations). Mouse VIIIA, which was not yet weaned at the time of inoculation, died on the tenth day. The picture it presented was identical to that seen in the younger mouse examined on the sixth day postinfection. The other three mice from litter VIII all died on the 11th day and also presented a similar appearance.

On the 17th day, the peritoneal washings of neither mouse contained free scolices, but masses similar to the above were present in both. The masses from the older mouse were invariably smoother, more dense and more difficult to tease apart than those of the younger. Those of the latter contained, for the most part, contractile, invaginated scolices which were swollen and in the process of vesicularization (Figures 5, 6 and 11). A single membrane (the first lamina?) surrounded each scolex. In the older mouse, most scolices were dead. A rare living scolex resembled those of the younger mouse.

On the 24th day, peritoneal washings from the younger mouse contained many swollen, vesicular scolices, most of which were surrounded by distinct, laminated membranes. Large numbers of these also comprised the numerous masses attached to the mesenteries, the spleen and free in the peritoneal cavity. Occasional nonvesicular scolices were also enclosed in laminated membranes. Peritoneal washings from the older mouse, on the other hand, contained many cells but no scolices. The numerous abdominal masses appeared darker and more dense than previously noted. They contained, almost exclusively, dead, black scolices (Figure 8). Occasional scolices were alive but none had vesiculated; one of normal size was surrounded by a distinct, laminated membrane (resembling forms seen by the authors in degenerating bovine hydatid cysts).

Mouse IA died on the 29th day; extensive masses, containing dead scolices, were applied to the diaphragmatic surface of the liver. The mice necropsied on the 33rd day resembled their counterparts on the 24th day.

Scolices in process of vesicularization in mice closely resemble those cultured in vitro (Schwabe, unpublished data). They first imbibe water and swell to several times the normal size. An explanation for this change is not apparent since the protonephridial flame cells, which are presumably excretory in function, continue to beat during this period. Accompanying this change is a migration of scolex cells to the periphery; these form the germinal membrane. All that remains of the central cells is a dense

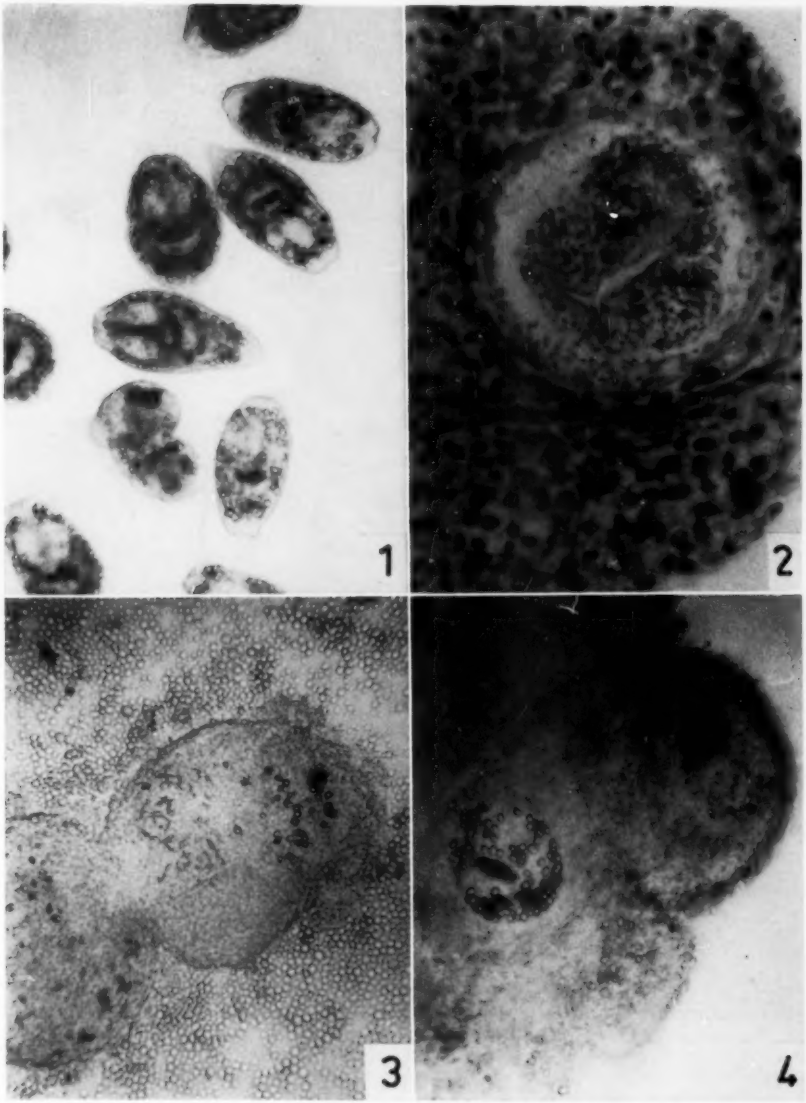


FIG. 1. 3 days postinfection, young mouse (6A), peritoneal washings. Normal invaginated scolices. ×290.

FIG. 2. 6 days postinfection, young mouse (6B), section through peritoneal mass. Normal scolex embedded in a mass of fibroblasts and heterophils. ×1,280.

FIG. 3. 3 days postinfection, old mouse (2A), peritoneal washings. Dead, degenerating scolices surrounded by host cells. ×260.

FIG. 4. 3 days postinfection, old mouse (2A), peritoneal mass teased apart. Dead scolices embedded in host cells. ×330.

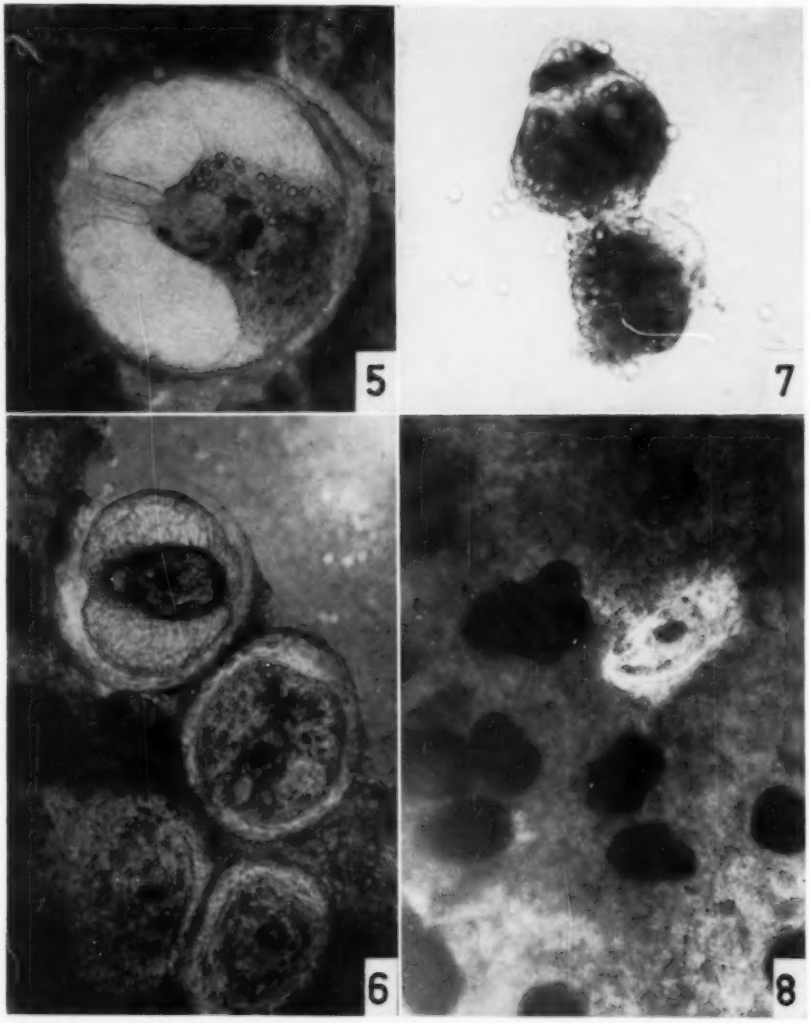


FIG. 5. 17 days postinfection, young mouse (6D), peritoneal mass teased apart. Vesiculating scolex. ×392.

FIG. 6. 17 days postinfection, young mouse (6D), peritoneal mass teased apart. Vesiculating scolices with the laminated membrane just beginning to form. ×276.

FIG. 7. 17 days postinfection, old mouse (2D), from teased peritoneal mass. Evaginated scolex, normal size, alive but beginning to degenerate. ×640.

FIG. 8. 24 days postinfection, old mouse (2E), peritoneal mass teased apart. Dead, black scolices, with a single, living scolex, embedded in host cells. ×260.

nucleus (the suckers and hooklets) connected to the surface layer by a few strands of cells and fibers. Microscopically the germinal surface of the young cyst presents a finely cross-hatched appearance.

By the 38th day postinfection, some individual cysts in the younger mouse were large enough to be visible to the naked eye; the older mouse contained only dense masses of host cells and dead scolices. On the 52nd day, the younger mouse

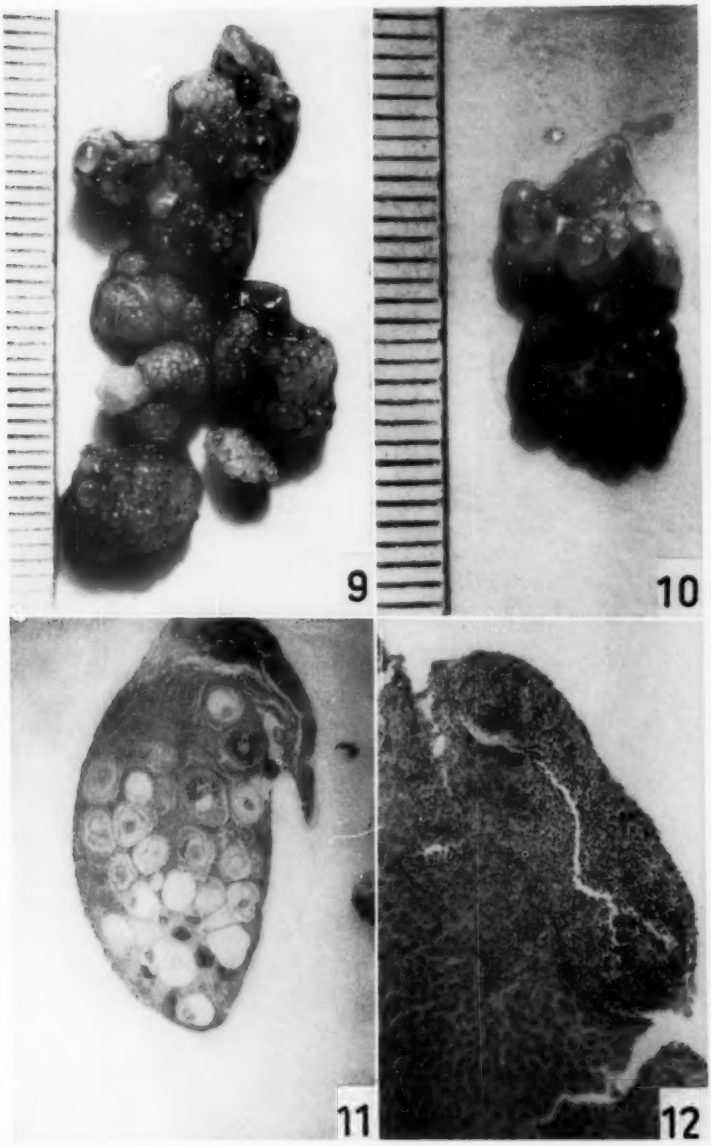
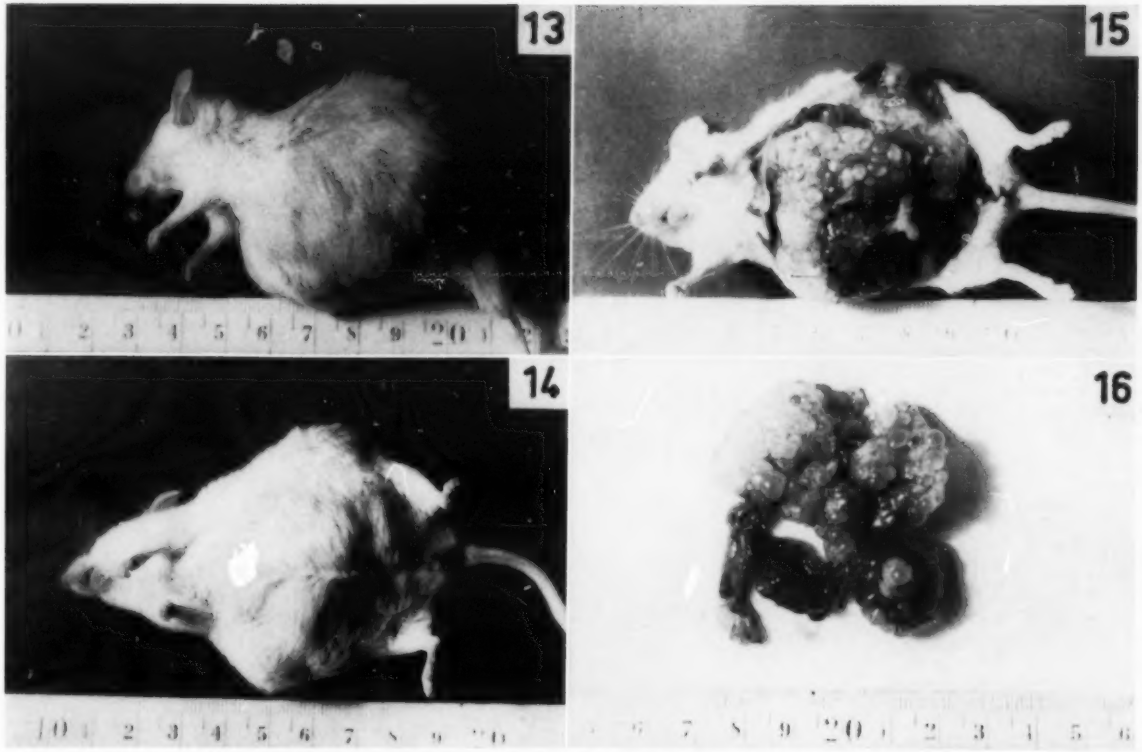


FIG. 9. 94 days postinfection, young mouse (7A), developing cysts removed from peritoneal cavity. Scale divisions 1 mm.

FIG. 10. 94 days postinfection, old mouse (1B), developing cysts removed from peritoneal cavity. Scale divisions 1 mm.

FIG. 11. 17 days postinfection, young mouse (6D), section through peritoneal mass. Young cysts embedded in fibroblasts and heterophils. ×96.

FIG. 12. 6 days postinfection, old mouse (2B), section through mass attached to liver. Dead, degenerated scolices embedded in fibroblasts and heterophils. ×270.



FIGS. 13 and 14. 105 days postinfection, young mouse (5E), abdomen enlarged by developing cysts. Smallest scale divisions 1 mm.

FIG. 15. 105 days postinfection, young mouse (5E) abdomen opened to show developing cysts. Smallest scale divisions 1 mm.

FIG. 16. 105 days postinfection, young mouse (5E), masses of developing cysts removed from peritoneal cavity. Smallest scale divisions 1 mm.

contained masses up to 2 cm in diameter attached to the intestines, spleen and liver. Discrete cysts were plainly visible to the naked eye. Masses contained very little host tissue and were almost entirely made up of developing cysts. Some of the latter were almost twice the size of those observed previously while others were still quite small. The largest cysts had the thinnest laminated membranes. The "nuclear masses" which contained the suckers and hooklets were still present, as were the "calcareous corpuscles" characteristic of normal scolices and other cestode tissues. On section, some of the cysts were seen to be almost completely surrounded by normal liver parenchyma which had begun to replace the host inflammatory tissue. The older mouse contained fewer masses, most of which were dense and small. These consisted of fibroblasts, heterophils and remnants of dead scolices. There were a very few developing cysts on the surface of the liver and some of these were larger than any in the younger mouse.

The mice necropsied on the 73rd and 94th days were not unlike their counterparts on the 52nd day (Figures 9 and 10). The numerous cysts in the younger mice and the few in the older mice continued to increase in size. Again, some in the older mice were larger than any in the younger. Vestiges of suckers and identifiable hooklets were still present. Some individual cysts were as large as 2.75 mm in diameter.

By the 105th day the abdomen of mouse VE was so distended (Figures 13 and 14) that it was decided to autopsy it out of schedule. The abdominal cavity, as in the other younger mice, contained many developing cysts (Figures 15 and 16), some as large as 7 mm in diameter.

The younger mouse autopsied on the 115th day also presented a much enlarged abdomen. Developing cysts were scattered in large numbers throughout the peritoneal cavity. Replacement of inflammatory tissue by normal liver parenchyma resulted in the inclusion of several cysts within the liver itself. Cysts consisted of the laminated and germinal membranes but none contained scolices or brood capsules. Even the largest still possessed a small cellular "nucleus" with "calcareous corpuscles" and hooklets. This small mass of cells and fibers is contractile; its contractability could serve as a criterion for viability in chemotherapeutic trials and other studies. The older mouse was of normal size. No developing cysts were found within its peritoneal cavity although some small yellow-white masses of host cells and scolex remnants were present, a few of which were embedded in the surface of the liver. The three younger mice remaining from Group VII and the last older mouse from Group I were necropsied on the 136th day. The abdomens of two of the younger mice were grossly distended by hundreds of developing cysts the largest of which measured 8 mm in diameter. While the abdomen of the third mouse was less distended, it also contained large numbers of developing cysts. By contrast, the older mouse was of normal size and proportions. Its peritoneal cavity contained four or five cysts which were each approximately the size of those in the younger mice.

The control mice from Group IV were also autopsied on the 136th day. None contained developing cysts, degenerate scolices or other evidences of infection.

DISCUSSION

It is sometimes stated in parasitology textbooks that children are more susceptible to echinococcosis than adults. Barnett (1945) remarked that, in most cases, a hydatid cyst is nearly as old as the patient. We have found little evidence in the literature to support such statements. In man, the disease is rarely diagnosed until several years after infection; one is therefore not likely to learn a great deal about age susceptibility from human data. The possible exception would be cysts of the brain which are more liable to early recognition. A literature survey recently completed by Dr. Fuad Haddad of the Department of Surgery of this University throws some light on this important question. He found that, of some 217 reported cases of echinococcosis of the brain, 138 (63.6%) were in children 15 years of age or less. This is suggestive of age resistance in man, although it may be argued, with some justification, that children are simply more likely to expose themselves to infection. Age resistance has, of course, been experimentally demonstrated for a number of other helminthiases, including the unique situation in *Neoascaris vitulorum* infection of cattle where most cases appear to be acquired prenatally (Refuerzo and Albis-Jimenez, 1954).

In earlier experiments with secondary echino-

coccosis in mice, Dévé (1933) and de Waele and de Cooman (1938) obtained 100 per cent infection in their series of animals, while Coutelin, Lecroart and Cochet (1939) succeeded in infecting only 65 per cent of inoculated mice. The latter authors attributed the difference in results to possible strain differences in the animals. No mention was made by these workers of the ages of the mice used. In preliminary experiments, we inoculated mice of the same strain, selected irrespective of age, with scolices from human, ovine and bovine sources and found that cysts developed in some of each group but not in others. With adequate attention to the age of mice it would now seem possible to infect this laboratory host routinely. The ease of establishing such laboratory infections in an inexpensive animal should provide a most useful situation for a variety of experimental studies on unilocular echinococcosis as well as for chemotherapeutic trials.

Host resistance to secondary echinococcosis in mice would appear to depend upon the rapid destruction of scolices by the host cellular reaction. Whether humoral factors are also involved is not yet known. The fact that the difference in susceptibility noted here occurred between mice 48 days of age or under and mice 71 days of age or older at time of inoculation is of possible significance. The age of sexual maturity in the laboratory mouse is given by Worden (1947) as 42 to 56 days. The data on human brain cysts already cited suggest further that sexual maturity may possibly be a factor in susceptibility to echinococcosis. Experiments now in progress in our laboratory are designed to test some of the possible relationships of the host endocrine system to natural resistance to this disease.

ACKNOWLEDGMENTS

The authors are grateful to Mr. John Himo for technical assistance. The human-brain hydatid was provided by Dr. Fuad Haddad, to whom we are also indebted for permission to use the data on human patients with hydatid cysts of the brain.

SUMMARY

1. White mice 48 days of age or younger at time of inoculation were highly susceptible to an initial infection with scolices of *Echinococcus granulosus* when the latter were given intraperitoneally.

2. Mice 71 days of age or older were relatively resistant to an initial infection under similar conditions.

3. Four mice which were unweaned at time of inoculation died on the 10th and 11th days postinfection.

4. The few cysts which developed in older mice were often larger than any which developed in younger counterparts. The possibility of an ecological "crowding effect" is suggested.

5. The difference in susceptibility to infection appeared to be related to the magnitude of the host's cellular response.

6. The development of hydatid cysts from scolices is described.

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HISTOPLASMIN, TUBERCULIN, AND COCCIDIOIDIN SKIN SENSITIVITY IN LIBERIAN ABORIGINES[1]

S. WARREN SEIDES, LAWRENCE R. BOIES, JR., AND STEPHEN E. MALAWISTA

Mochi and Edwards in 1952 discussed the incidence of histoplasmin sensitivity in different parts of the world and emphasized the need for a more thorough, world-wide study of sensitivity rates, especially in Africa. Although recently several investigators have reported surveys from Africa, large regions of this vast continent remain unstudied.

The Republic of Liberia, located on the tip of the great West African bulge, lies between four and eight degrees north of the Equator. Rainfall averages from 180 inches annually along the coast to 70 inches inland. The population of Liberia is estimated to be 750,000. More than 95% of these people are aborigines; members of the many tribes which have inhabited the country for centuries. The remainder are Americo-Liberians, descendants of the freed American Negro slaves who founded this republic in the early 1800's.

The purpose of this survey is to determine the frequency of histoplasmin sensitivity in conjunction with tuberculin and coccidioidin sensitivities in Liberia; one of the regions unstudied in this regard.

MATERIALS AND METHODS

Firestone Rubber Plantation, located 10 miles from the coast, occupies more than 90,000 acres. Firestone's 30,000 aboriginal male employees migrate with their families to and from their individual tribal villages in the bush to reside in mixed communities on the plantation.

The present survey was carried out on 1,031 apparently healthy, male rubber tappers and 248 school children (33 females, 215 males). Sixteen different tribes were represented. Among these the Fanti are coastal dwellers and fishermen. The Mandingo are peripatetic traders. The remaining tribes live near the Liberian rivers. The working natives do not know their ages, although the range was approximately 10 to 75 years old, as judged by the authors and their native assistants. The ages of the school children were known, and ranged from 4 to 18 years.

Both histoplasmin (Parke, Davis & Company) 0.01 mg and coccidioidin (Cutter Laboratories) 0.1 mg, in 0.1-ml dosages, were administered intracutaneously in the left forearm. Old tuberculin 0.01 mg was given in a dose of 0.1 ml in the right forearm.

Separate syringes and needles were used for each test agent. All equipment (except needles) was cleaned, prior to skin testing, with 50 g of sodium dichromate in solution with 1 L of concentrated sulfuric acid, and then autoclaved.

Histoplasmin and tuberculin reactions were read after 48 and 72 hours; coccidioidin after 24 and 48 hours. Induration of 5 mm or more was the sole criterion of positivity in all three tests.

RESULTS

Histoplasmin sensitivity. A heretofore unrecognized incidence of 16.8 per cent skin sensitivity to histoplasmin appeared in the overall sample of 778 persons tested. Whereas 712 male workers produced 129 positive reactions (18.1%), only 3.0 per cent of 66 children gave positive results (Table 1).

Because of an age-group overlap between the oldest school children (ages 10 to 18 years) and the youngest tapping boys (estimated to be less than 20 years old), the children's group was subdivided into two groups (Table 2). The first group of 33 children between 4 and 9 years old produced no positive skin tests. The second group of 33 children between 10 and 18 years old showed two positive reactions. There seems to have been a slightly higher incidence of reactivity in the group of workers estimated to be under 20 years old (11.3%) than in the second group of school children (a comparable age group showing 6.1% positivity). However, no statistical comparisons are made because the group of school children provides too small a sample, and because the young workers were not an accurately determined age group.

Table 3 shows that 4.4 per cent of the 778

[1] From the Department of Medicine and the School of Public Health and Administrative Medicine, College of Physicians and Surgeons, Columbia University, New York and the Medical Department, Firestone Hospital, Harbel, Liberia, West Africa.

TABLE 1

Incidence of skin sensitivity in Liberians

	Histoplasmin			Tuberculin			Coccidioidin		
	No.	No. pos.	% pos.	No.	No. pos.	% pos.	No.	No. pos.	% pos.
Workers	712	129	18.1	1031	770	74.7	717	9	1.3
Children	66	2	3.0	248	95	38.3	16	0	0.0
Totals	778	131	16.8	1279	865	67.6	733	9	1.2

TABLE 2

Incidence of skin sensitivity of Liberian children

Children's age groups	Histoplasmin			Tuberculin			Coccidioidin		
	No.	No. pos.	% pos.	No.	No. pos.	% pos.	No.	No. pos.	% pos.
4–9 yr (school)	33	0	0.0	74	18	24.3	10	0	0.0
10–18 yr (school)	33	2	6.1	174	77	44.3	10	0	0.0
<20 yr (workers — of 712 adult males)	106	12	11.3	106	44	41.5	—	—	—

TABLE 3

Skin reactivity changes between first and second readings

	Histoplasmin (778)		Tuberculin (1,279)		Coccidioidin (733)	
	Changed	% change	Changed	% change	Changed	% change
Pos. at 48 hr, neg. at 72 hr	13	1.7	37	2.9	7	0.9
Pos. at 72 hr, neg. at 48 hr	21	2.7	47	3.7	2	0.3
Total change	34	4.4	84	6.6	9	1.2

people had significant histoplasmin induration at only one reading, 1.7 per cent of these at 48 hours and the remaining 2.7 per cent at 72 hours.

Tuberculin sensitivity. Of 1,031 male rubber tappers ranging in age from approximately 10 to 75 years, 770 (74.7%) were found to have induration of 5 mm or greater in diameter (Table 1). Ninety-five of 248 school children (38.3%) had positive skin sensitivity. Thus, the entire tested population of 1,279 individuals revealed a 67.6 per cent rate of positivity.

Of the 74 children in the 4- to 9-year age group 24.3% showed positive reactivity. Of the 174 older school children 44.3% gave positive reactions. This latter figure compares favorably with the 41.5% sensitivity rate in male workers judged to be less than 20 years old (Table 2). We thus gain the impression of a directly proportional relationship between positive skin sensitivity and age.

Table 3 shows that 6.6 per cent of the 1,279 people tested had positive reactions at only one reading, 2.9 per cent of these at 48 hours and the remaining 3.7 per cent at 72 hours.

Coccidioidin sensitivity. Nine (1.2%) of 733 subjects reacted positively to coccidioidin. All of these had transitory reactions, showing positivity at only one reading (Table 3). None of 16 children's reactions reached 5 mm of induration.

Tribal distribution. The tribal distribution of skin sensitivity to each of the three test agents used is presented in Table 4. The Kpelle tribe exhibited a low incidence of positivity to histoplasmin (12.4%), whereas both the Gissie and Gio tribes showed 28.8 per cent and 26.3 per cent, respectively. These differences should be investigated further for statistical and etiological significance.

Cross reactivity. Table 5 presents a comparison of the sizes of tuberculin and histoplasmin reactions. A relationship between these reactions may occur by chance as much as 22 per cent of the time (χ^2 test). Thus, there is no statistical evidence of cross reactivity between the two test agents.

DISCUSSION

Since Mochi and Edwards emphasized the need for epidemiological studies of histoplasmosis in 1952, several such studies have been carried out and were reviewed by Edwards and Kiaer in 1956. The incidence of sensitivity to histoplasmin varied from 5 per cent to 15 per cent in samples from the general population of Algeria, Belgian Congo, French Equatorial Africa, Kenya, Sudan, Uganda, and the Union of South Africa. A study done along the Nile River in Egypt revealed no positive reactors. The variation in sensitivity rates led Edwards and Kiaer to stress the idea that the incidence of sensitivity in a local sample cannot be applied to a whole

TABLE 4

Tribal distribution of skin sensitivity

Tribe	Histoplasmin				Tuberculin				Coccidioidin			
	Workers		Children		Workers		Children		Workers		Children	
	No.	% pos.	No.	% pos.	No.	% pos.	No.	% pos.	No.	% pos.	No.	% pos.
Kpelle	178	12.4	13	7.7	223	74.4	41	34.1	176	1.1	5	0
Mano	149	16.8	3	0	260	68.8	10	60.0	149	0.7	—	—
Gissie	95	28.8	6	0	116	80.2	12	25.0	96	1.0	1	0
Gio	95	26.3	3	0	141	73.0	16	25.0	96	3.1	—	—
Bassa	54	11.1	20	0	83	78.3	73	50.7	57	1.8	3	0
Buzi (Loma)	36	8.3	6	0	50	82.0	26	23.1	36	2.8	1	0
Gbandi	31	22.6	2	0	45	66.7	10	70.0	31	0	—	—
Belle	21	19.0	—	—	25	84.0	—	—	20	0	—	—
Krahn	19	21.5	1	100	34	70.6	5	20.0	21	0	—	—
Mendi	19	15.8	3	0	26	100	6	16.6	19	0	1	0
Gola	7	42.9	1	0	17	82.4	8	37.5	7	0	—	—
Gribo	6	0	1	0	6	83.3	3	66.7	6	0	—	—
Kru	1	0	5	0	2	100	20	25.0	1	0	4	0
Mandingo	—	—	—	—	2	50.0	1	100	—	—	—	—
Vai	—	—	2	0	—	—	14	35.7	—	—	1	0
Fanti	—	—	—	—	—	—	3	0	—	—	—	—
Total	711	18.1	66	3.0	1030	74.8	248	38.3	715	1.3	16	0

TABLE 5

Relationship of the extent of tuberculin and histoplasmin reaction*

Tuberculin reaction	Histoplasmin reaction			
	<5 mm (neg.)	5–10 mm	>10 mm	Totals
<5 mm (neg.)	164	9	18	191
5–10 mm	108	12	7	127
11–20 mm	234	27	34	295
>20 mm	77	8	14	99
Totals	583	56	73	712

* $\chi^2 = 8.98$, d = 6, p = 0.22.

country. In Sudan Edwards systematically covered the entire country, testing a total of 6,000 persons. The incidence of sensitivity was less than 2 per cent in most areas, but in the province of Equatoria an incidence of 15 per cent was found.

The adult males in the present study represent a migrant labor force with an undefined rate of turnover, largely from four tribes. These workers provided a random sample of members of Liberia's tribes intermingling in one locale. A more representative sample of the country's male population could perhaps be obtained by testing in individual tribal villages.

Histoplasmosis has been known to exist in Liberia. Postmortum tissue diagnoses of pulmonary histoplasmosis were made on two patients at Cape Palmas, Liberia, whose deaths were attributed to lung lesions. However, to our knowledge this is the first population sample tested for histoplasmin sensitivity in Liberia.

Examination of injection sites at both 48 hours and 72 hours is shown to be a profitable technique, in both histoplasmin and tuberculin studies (Table 3). Errors in reading undoubtedly play a role here, as well as actual waxing or waning of induration.

In Africa, as in many parts of the world, sensitivity to tuberculin runs high, with positives varying between 50 per cent and 90 per cent, depending on age, locality, and testing method employed. Our figure of 74.7 per cent positivity in Liberia is not surprising, as clinical tuberculosis is frequent on the plantation. In contrast, the prevalence of histoplasmosis had been unknown. The finding that 18.1 per cent of the adult group were skin-positive is striking

in that the incidence of sensitivity to histoplasmin is thus roughly one quarter that of sensitivity to tuberculin. This means that, on the basis of a skin test, as many as one out of every five patients with apparent pulmonary tuberculosis might actually have pulmonary histoplasmosis.

We know of no studies of coccidioidin sensitivity in West Africa. A study in South Africa failed to reveal such sensitivity. The 1.3 per cent positive reactions found in the nine persons tested in our study were probably due to nonspecific causes, for the following reasons: (1) Coccidioidomycosis is a highly infectious disease. Littman, *et al.*, 1958 make the statement that "even limited exposure to infected dust during brief travel through an endemic area may be sufficient to cause infection." The nine positives found in this study represent the six tribes which have the largest representation in the sample. If their tribal regions were endemic foci, more members of these tribes should have had positive coccidioidin tests. An alternative hypothesis, and a doubtful one, assumes an endemic area, presumably in Liberia, through which the positive persons wandered at some time, contracting the disease. (2) None of the nine positive coccidioidin tests was positive at both 24 and 48 hours. The greatest induration reached was 8 mm. (3) Tuberculin tests were positive in all of the nine persons with positive coccidioidin tests. One also had a positive histoplasmin reaction.

SUMMARY

1. The incidence of skin sensitivity to histoplasmin among 712 working Liberian males was found to be 18.1 per cent and among 66 school children 3.0 per cent. Of the tested population 4.4 per cent showed positive reactions at only one of the two readings taken. Distribution of reactivity among 16 Liberian tribes is presented.

2. The incidence of positive tuberculin tests among 1,031 working Liberian males was 74.7 per cent, and among 248 school children 38.3 per cent. Of the total tested population 6.6 per cent reacted positively at only one reading. There was no significant cross reactivity between the histoplasmin and tuberculin agents used.

3. The incidence of positive skin tests to coccidioidin in 733 subjects was 1.2 per cent.

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VIRUS TRANSMISSION STUDIES WITH TRINIDADIAN MOSQUITOES

PART II. FURTHER OBSERVATIONS[1]

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The list of Trinidadian arthropod-borne viruses isolated in the past includes the following known agents: yellow fever, dengue, Venezuelan equine encephalitis, Ilhéus, St. Louis and Mayaro (Downs *et al.*, 1955; Anderson, Aitken and Downs, 1956; Anderson, Downs and Hill, 1956; Anderson, Aitken *et al.*, 1957; Anderson, Downs *et al.*, 1957; Gilyard, 1944, 1945). Of these, only the latter three appear to be presently active in the human or animal population. The yellow fever epidemic of 1954 subsided with the mass immunization of human beings, mosquito control operations and the presumed build-up of immunity in the wild-monkey population. Operations to eradicate *Aedes aegypti* appear to be influencing the incidence of dengue, as no cases have been seen since 1954. Likewise, there have been no observed outbreaks of Venezuelan equine encephalitis since the one experienced in 1943–44, nor have immunological surveys disclosed any recent infections attributable to this disease (Downs, Anderson and Theiler, 1956).

On the other hand, in every year since 1954 Ilhéus virus has repeatedly been isolated by this laboratory from mosquitoes as well as several times from human beings (Downs, Anderson *et al.*, 1956, and unpublished information). St. Louis virus was isolated from mosquitoes in 1955 and from a nestling dove in 1956 (Anderson, Aitken *et al.*, 1957; Downs *et al.*, 1957). Finally, Mayaro virus, a new agent obtained from human beings in south Trinidad in 1954 (Anderson, Downs *et al.*, 1957) has been found occurring naturally in forest mosquitoes in 1957 (in preparation).

As part of the general study of anthropod-borne viruses in Trinidad, studies were initiated to determine the infectibility of local mosquitoes. Preliminary experiments with the currently active viruses of Ilhéus, St. Louis and Mayaro were discussed in general terms in a previous communication (Aitken, 1957). A more detailed discussion of a larger number of experiments forms the subject of this report.

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the Government of Trinidad and Tobago, the Colonial Development and Welfare Scheme and The Rockefeller Foundation.

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MATERIALS AND METHODS

Viruses. Ilhéus: TRVL 5800 (2nd passage) isolated from a mixed pool of Trinidadian mosquitoes (Anderson, Aitken and Downs, 1956); St. Louis: TRVL 9464 (6th passage) isolated from Trinidadian *Psorophora ferox* (Anderson, Aitken *et al.*, 1957); and Mayaro: TRVL 4675 (4th passage) isolated from a Trinidadian forest worker (Anderson, Downs *et al.*, 1957).

Diluent. The diluent in which mosquitoes were triturated, is composed of 0.12 molar NaCl; 0.006 molar NaH_2PO_4; 0.012 Na_2HPO_4; and 0.75% fraction-5 bovalbumin. The pH is adjusted to between 6.8 and 7.3 by the addition of 0.3 molar NaOH solution. Sterilization is effected by filtration through a Seitz EK pad. Crystalline penicillin G and streptomycin sulphate are added to produce a final concentration of 400 units per ml of the former and 250 μg per ml of the latter.

Mosquitoes. Ten species of mosquitoes were infected: *Aedes aegypti*, *A. scapularis*, *A. serratus*, *Culex quinquefasciatus*, *Mansonia arribalzagai*; *M. venezuelensis*, *M. wilsoni* (questioned identity, *titillans* of Trinidadian literature), *Psorophora albipes*, *P. ferox* and *Trichoprosopon digitatum*. The *Culex* and *Aedes aegypti* came from laboratory colonies and were used when 4 to 8 days old (*Culex*) and 7 to 14 days (*Aedes*). *Trichoprosopon digitatum* larvae were brought in from the field and reared. Adults 2 to 3 days of age were employed. The remaining species were caught as adults in the forest, their larvae being unobtainable or else failing to develop in the laboratory. As a consequence of their exposure

to the rigors of collecting, and also because of varying age, considerable mortality was experienced in this group.

Infection of mosquitoes. Mosquitoes were infected parenterally by inoculating anesthetized specimens in the thorax (membranous area below wing base) either (1) with a *minuten nadeln* previously dipped in the virus suspension (Note: two needles were used alternately and flamed after each operation), or (2) with a fine glass capillary. The Ilhéus inoculum was a 20-per-cent-brain suspension which titered between $10^{-6.3}$ and $10^{-7.5}$; that of St. Louis 10 per cent and 20 per cent, titering $10^{-6.3}$; and that of Mayaro a 1 and 10-per-cent-brain suspension, titering between $10^{-5.8}$ and $10^{-6.7}$. Mosquitoes were anesthetized by exposing them in groups of five to CO_2 for 1 to 2 minutes. Following inoculation they were placed in bobbinet-covered, cylindrical, plastic cages (3 × 4 inches) provided with cotton pledgets of sugar solution. Average maximum and minimum temperatures of the storage room were 81° and 77°F (extremes 88-68°F).

Immediately following the mosquito inoculations (2 hours or less after the start of these operations), the virus suspension was titrated in Swiss mice. Originally this was done both before and after mosquito inoculations, but as no fall in virus titer was noted (virus was kept iced constantly), the first titration was eliminated.

Titrations were performed either in groups of six adult mice (generally 28–35 days old) when using Ilhéus or St. Louis virus (0.03 ml per mouse, intracerebrally = i.c.), or else in groups of six to eight 2-day-old mice (0.03 ml intraperitoneally or 0.02 ml i.c.) for Mayaro virus. The 50-per-cent endpoints were calculated by the method of Reed and Muench (1938).

TABLE 1

Laboratory transmission of Ilhéus virus by inoculated Trinidadian mosquitoes

Mosquito	Incub. period (*days*)	No. of positive mosquitoes feeding	Virus transmission				
			Chicks				Mice No. pos./ no. bitten
			Circ. virus (Mort. ratio)		Antibody production		
			P-i.-day 3	P-i.-day 4	Test dose (MLD 50)	Mort. ratio	
Aedes aegypti	6	1	4/6 (P)*	5/6 (P)	100	5/6 (N)	
	6	5	4/6 (P)	6/6 (P)	Chick died	—	
A. scapularis	7	1, 2					2/2
	8	1					1/1
	10–11	7	5/6 (P)*	4/6 (P)	262	2/6 (PP)	
	10–11	8	5/6 (P)*	4/6 (P)	262	3/6 (PP)	
	11	1, 1, 1, 2, 2					5/5*
	12	1					1/1*
	13	1					1/1
	14	1, 1					2/2
A. serratus	7	1					0/1
	10	1 per mouse					2/6*
Culex quinquefasciatus	9	2	5/6 (P)*	6/6 (P)	Chick died	—	
	9	1	1/6 (P)	6/6 (P)	251	3/6 (PP)	
	9	1	4/6 (P)	5/5 (P)*	100	5/5 (N)	
	9	1	6/7 (P)*	6/6 (P)	251	6/6 (N)	
	10	3	—	6/6 (P)*	251	6/6 (N)	
	11	1	3/6 (P)*	4/6 (P)	262	3/6 (PP)	
	11	1 per mouse					3/3*
	12	1, 1, 2, 3, 3, 3					6/6*
	14	1, 1, 1, 2, 2					5/5*
Psorophora albipes	10	1	5/6 (P)*	2/6 (P)	Chick died	—	
P. ferox	10	1, 1 (?)					1/2*

* Virus confirmed by specificity test; P = positive, PP = partial positive, N = negative.

At intervals following exposure to infection, mosquitoes (dead, or anesthetized with CO_2) were ground individually (rarely pools of two or more mosquitoes) in 1 ml of bovalbumin diluent, spun in an angle centrifuge at 8,000 rpm for 10 minutes, and the supernatant fluid inoculated into mice to test for presence of virus. Brain suspensions (10% in saline) of sick mice were passaged i.c. to groups of fresh mice until a typical infection resulted. Virus (brain material) from one or more of such typical infections in a particular experiment was then set up in a test with normal and immune sera to the virus in question to establish its identity.

Likewise at times, infected mosquitoes, either singly or in groups, were permitted to bite 1- to 2-day-old domestic chicks and 2-day-old mice. Brains of sick mice were removed for passage to healthy mice as indicated above. To demonstrate circulating virus in chicks, they were bled (0.1–0.4 ml) from the neck vein at 3- and 4-day intervals following exposure to mosquitoes; the blood was diluted with saline or bovalbumin, at most 1:4, centrifuged and inoculated i.c. into mice. One month following exposure to infected mosquitoes, any surviving chicks were exsanguinated and the serum tested for specific antibody by neutralization test.

RESULTS

The results of experiments with various species of mosquitoes are summarized in Tables 1, 2 and 3. *Only studies with virus-positive mosquitoes are recorded.*

Ilhéus (Table 1). Transmission was effected by all species tested after incubation periods varying from 6 to 14 days, by single individuals (26 times) as well as groups of two to eight mosquitoes. One of two attempts to transmit by single *Psorophora ferox* failed, but it was extremely doubtful if the specimen fed on the one occasion. It is of interest to note that in several instances chicks failed to demonstrate specific antibodies on being bled 1 month after circulating virus had been recovered.

St. Louis (Table 2). All of the species biting test animals likewise transmitted St. Louis virus. The incubation period was somewhat short, which may account for some transmission failures on the part of *Aedes serratus* and *Psorophora ferox*; however, three of the four successes with *Mansonia arribalzagai* occurred

TABLE 2

Laboratory transmission of St. Louis virus by inoculated Trinidadian mosquitoes

Mosquito	Incub. period (days)	No. of positive mosquitoes feeding	Virus transmission				
			Chicks				Mice No. pos./ no. bitten
			Circ. virus (Mort. ratio)		Antibody production		
			P-i.-day 3	P-i.-day 4	Test dose (MLD 50)	Mort. ratio	
Aedes aegypti	9	1	6/6 (P)*	4/6 (P)	2,541	2/7 (P)	
	9	3	6/6 (P)*	3/5 (P)	2,541	0/7 (P)	
A. serratus	9	1	0/5 (N)	Chick died	—	—	
	9	3	6/6 (P)*	1/6 (?P)	2,541	3/7 (PP)	
Culex quinquefasciatus	8	1	5/6 (P)*	6/6 (P)	157	1/7 (P)	
	11	1					1/1 (P)*
Mansonia arribalzagai	7	2	5/5 (P)*	6/6 (P)	Chick died	—	
	7	1–2 probed	3/6 (P)	6/6 (P)*	Chick died	—	
	7	2	6/6 (P)*	6/6 (P)	157	0/7 (P)	
	10	1	—	0/6 (N)	Chick died	—	
	10	1	—	6/6 (P)*	Chick died	—	
Mansonia venezuelensis	7	1	6/6 (P)*	5/6 (P)	157	0/7 (P)	
Psorophora ferox	7	1	6/6 (P)*	6/6 (P)	Chick died	—	
	9	1	0/6 (N)	0/6 (N)	Chick died	—	
	9	1	0/6 (N)	0/6 (N)	2,541	7/7 (N)	

* See footnote to Table 1.

TABLE 3

Laboratory attempts to transmit Mayaro virus by inoculated Trinidadian mosquitoes

Mosquito	Extrinsic incub. period (*days*)	No. of positive mosquitoes feeding	Virus transmission			
			Chicks			
			Circ. virus (Mort. ratio)		Antibody production	
			P-i.-day 3	P-i.-day 4	Test dose (MLD 50)	Mort. ratio
Aedes scapularis	7	3	7/7 (P)*	7/7 (P)	373	2/7 (P)
	9	1	0/7 (N)	0/6 (N)	653	7/7 (N)
	9	1	0/7 (N)	0/5 (N)	653	7/7 (N)
	9	1	—	0/7 (N)	653	7/7 (N)
	10	1	0/6 (N)	0/6 (N)	653	7/7 (N)
	10	1	0/5 (N)	0/7 (N)	653	7/7 (N)
	10	1	0/6 (N)	0/6 (N)	653	7/7 (N)
A. serratus	9	1	0/7 (N)	0/6 (N)	653	7/7 (N)
Culex quinquefasciatus	7	7	0/7 (N)	0/7 (N)	Chick died	—
	7	1	0/7 (N)	0/7 (N)	Chick died	—
	7	12	0/7 (N)	0/7 (N)	Chick died	—
	12	1	0/7 (N)	0/7 (N)	Chick died	—
	13	1	0/7 (N)	0/7 (N)	373	6/6 (N)
Mansonia wilsoni	6	1	0/7 (N)	0/7 (N)	373	5/5 (N)
	8	2	0/7 (N)	0/7 (N)	Chick died	—

* Virus confirmed by specificity test; P = positive, N = negative.

after an incubation period of only 7 days. Single mosquitoes were responsible for transmission by all species except *Aedes serratus*. In the latter instance, three specimens effected the transmission, and in two of the experiments with *Mansonia arribalzagai*, two individuals in each instance engorged on the recipient animals. Antibody production was demonstrated in all chicks who survived 1 month following exposure to infected mosquitoes and in whose blood circulating virus had been demonstrated.

St. Louis virus was recovered from three *Trichoprosopon digitatum* females when these were ground at 7, 9 and 11 days after inoculation. Attempts to induce this species to feed failed.

Mayaro (Table 3). A single transmission, by one *Aedes scapularis* after an incubation period of 7 days, has thus far been obtained. Circulating virus recovered on the third postinfection date from the recipient chick was identified by specificity test as Mayaro virus (being protected by more than 2.7 logs of immune serum). Convalescent serum collected 1 month later showed evidence of neutralizing antibody to Mayaro virus by protecting 5 of 7 mice ina neutralization test where the dosage was 373 mouse LD_{50}'s.

Six other inoculated species have harbored Mayaro virus for at least 12 days. These are: *Aedes serratus, Culex quinquefasciatus, Mansonia arribalzagai, M. venezuelensis, M. wilsoni* and *Psorophora ferox*.[2] Of these only *A. serratus, C. quinquefasciatus* and *M. wilsoni* lived long enough to be used in biting experiments, and none of them transmitted. As many as seven infected *quinquefasciatus* on one occasion, and 12 on another, engorged on single chicks at 7 days without transmitting; a single specimen did not transmit at 12 days. Possibly a longer incubation period or another host animal is required by this strain of Mayaro virus.

DISCUSSION

In certain instances these transmissions may take on added significance when compared with results obtained from grinding naturally-infected mosquitoes brought in from the field. Evidence is accumulating which shows that

[2] In a different type of experiment, three *Mansonia arribalzagai* and one *M. venezuelensis* became infected after feeding on cotton pledgets impregnated with a 20 per cent suspension of virus. None, however, transmitted virus to day-old chicks after an incubation period of 9 to 10 days.

certain species of ground-breeding *Psorophora* and *Aedes* may be the important local vectors of Ilhéus virus. Of nine virus isolations from mosquitoes to date, two came from pure pools of *Psorophora ferox* and one from *Aedes scapularis*. Two additional strains stemmed from *Psorophora* pools, mainly containing *P. ferox*, and the remaining four strains came from pools in which *P. ferox*, *A. scapularis* and *A. serratus* predominated.

Only one of the three species of mosquitoes from which naturally-occurring St. Louis virus was isolated has been successfully tested in the laboratory. *Psorophora ferox* transmitted once in three trials. Attempts were made to establish a colony of *Culex coronator*, but these met with failure when the adults refused to feed even though a variety of animals were offered them. *Culex caudelli*, the third naturally-infected species, is very small and attempts to inoculate the adults met with no success.

Little can be said about Mayaro virus at this time, since only one laboratory transmission has thus far been effected and the species involved is not the same as the one found infected in nature. Additional studies are required, involving a longer extrinsic incubation period and a host other than the chick.

SUMMARY

Evidence is presented that, with techniques of parenteral inoculation of virus followed by later feeding on susceptible chicks or mice, six species of Trinidadian mosquitoes transmitted Ilhéus virus, six species transmitted St. Louis virus and one species transmitted Mayaro virus.

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A BENTONITE FLOCCULATION TEST FOR THE DIAGNOSIS OF HYDATID DISEASE IN MAN AND ANIMALS

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As part of a more comprehensive study of the laboratory diagnosis of hydatid disease and the epidemiology of the disease in North America (Sadun, *et al.*, 1957), the bentonite flocculation (BF) test described by Bozicevich *et al.*, (1951) for trichinosis has been adapted and evaluated as a serological technique for the diagnosis of echinococcus disease in man and animals. The results obtained with 6,689 serum samples collected over a period of 3 years are here reported. They are compared with the results obtained by complement-fixation (CF) tests using the same antigens. In addition, several types of antigens were prepared and compared with a standard hog-hydatid-fluid antigen.

MATERIALS AND METHODS

In the BF test, antigen is adsorbed onto standard-size bentonite particles which, after adjustment to eliminate spontaneous saline flocculation, are added to serial dilutions of serum on a wax-ring slide. After mechanical rotation for 15 minutes, the dilutions showing more than 50 per cent of the particles aggregated into floccules are read as positive. The performance of the test and the reagents used were the same as previously described for trichinosis tests (Sadun and Norman, 1955) except for the antigens used. All of the sera were tested by this method.

Two types of antigens were prepared for adsorption onto bentonite. Hydatid-fluid (HF) antigens were concentrated from hog, human and horse[2] echinococcus cysts, and scolices antigens were extracted from hydatid sand collected from hog cysts. Preparation of the HF antigens described previously (Sadun *et al.*, 1957) consisted of the dialysis of the fluid to remove the salts, and concentration by perevaporation to approximately 0.33 of original volume. Isotonicity was restored by addition of NaCl. Scolices extracts were prepared in three ways. For type A antigen, 2 ml of washed, packed (by centrifugation at low speed) hydatid sand were triturated with 40 ml of neutral physiological saline and permitted to extract for 24 hours at 4°C. Insoluble material was removed by centrifugation at high speed for 30 minutes, and the extract adjusted to neutral if necessary. In type B antigen, 0.4 g of lyophilized, washed hydatid sand was substituted for the wet material. Type C antigen was made according to the directions of Chaffee *et al.*, (1954) for the preparation of fat-free trematode antigens. The procedure was essentially the same as for type B except that the lyophilized hydatid sand was extracted with cold anhydrous ether prior to trituration in saline. All antigens were preserved with Merthiolate 1:10,000 and stored at 4°C or frozen. Since hog HF antigen has been used routinely for 10 years in the diagnostic serology laboratory, it was chosen as the standard antigen for all comparisons. The Kjeldahl nitrogens on various batches of this antigen have varied slightly around 0.1 mg N/ml.

Positive control sera for all tests were obtained from a limited number of proved human cases. In addition, antisera prepared against the various antigens by inoculating rabbits intramuscularly with $Al(OH)_3$-precipitated antigens (Dorin, 1946) were used as controls.

The human sera tested were obtained from several sources: 413 were from symptomatic cases sent to the laboratory for diagnosis of hydatid disease. Of these sera, 18 were known to be from surgically verified cases. Histories on the others were not available. One hundred forty five sera were from people ill with other diseases including tuberculosis, trichinosis, and histoplasmosis. The remaining specimens were collected in surveys on 6 American Indian reservations in the United States, and from Eskimos and military personnel stationed in Alaska. A group

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[2] The horse hydatid fluid antigen was kindly supplied by Dr. J. H. Bekker, National Institutes of Public Health, Utrecht, The Netherlands.

of 197 sera from healthy Army and Air Force men in the United States served as negative control sera.

Over 1,700 specimens of serum were collected from 12 species of wild animals trapped in Georgia and Florida (Brooke *et al.*, 1957). In addition, 222 sera were from laboratory and domestic animals before and after immunization with various bacterial, viral, and parasitic antigens (except cestode antigens).

The CF test was performed by the 50 per cent Kolmer technique (as described by Bozicevich *et al.*, 1946) in the serology unit of our organization. Both hog HF and human HF antigens were used. A total of 4,148 of the sera were tested by this method.

RESULTS

The sources of the human sera tested and the results obtained by BF and CF tests are summarized in Table 1. None of the sera from normal individuals was positive by either test. Low flocculation titers were obtained in 5.5 per cent of the sera submitted for diagnosis of other diseases. Although the particular group of sera included in the table was not tested by CF, previous experience has shown that only very rare sera, even when serologically positive with other antigens, react with the echinococcus antigen in the CF test.

Of the 413 sera that were submitted for diagnosis of possible echinococcus disease, 42 were positive by BF and 20 by CF. Fourteen were positive by both tests. The highest titers were found in this group of sera. Of all the sera, the titers by BF varied between 1:5 and 1:2,560, and by CF from 1:4 to 1:128. However, the majority of the titers in all but the "symptomatic" group were 1:5 to 1:10 by BF.

The Alaskan sera were from individuals who may have been exposed to either *Echinococcus granulosus* or *E. multilocularis*. The serologically-positive sera (3.5 per cent by BF) were almost exclusively from Eskimos.

The percentage of positive BF reactions varied from 1.3 among 2,163 Utah Indians to 12.1 among 861 Indians from five other reservations. In contrast, the highest percentage of positives by CF was 4.9 in the symptomatic-cases group. The rates obtained by both tests were high in the American Indian group and, more specifically, in the sera from the Arizona and New Mexico reservations. Fecal examinations from samples of these populations did not show an increased incidence of intestinal helminth infections. However, more sheep are raised in these areas. Since tuberculosis rates were very high among all the Indians, a control group of sera from 45 white people in a tuberculosis sanitarium were tested by the BF test. Three sera (6.6%) were positive with the echinococcus antigen with titers of 1:5 or 1:10.

Altogether, 2,406 human sera were tested by both tests using the same lots of antigen. Two hundred eight (8.6%) were positive by one or both tests. There were 158 (6.6%) positive by BF and negative by CF; 26 (1.1%) positive by CF only, and 24 (1.0%) positive by both tests. Included in the 2,406 specimens were 18 samples from surgically-proved cases of hydatid disease. The results of the 2 tests on the 208 sera giving positive reactions are shown in Table

TABLE 1

Reactions obtained by BF and CF tests on human sera

Source of serum	No. of sera	Positive by BF		Positive by CF	
		No.	%	No.	%
U. S. normal individuals	197	0	0	0	0
U. S. non-symptomatic cases but ill with other diseases	145	8	5.5	Not tested	
U. S. symptomatic cases submitted for diagnosis of hydatid disease	413	42	10.1	20	4.9
Alaska military personnel and Eskimos	935	33	3.5	6	0.6
U. S. Indians. Utah	2,163	28	1.3	Not tested	
U. S. Indians. 5 Reservations	861	105	12.1	25	3.0
Totals	4,714	216	4.6	51	2.1

TABLE 2

Human sera showing positive reactions by BF and/or CF tests

Source of sera	No. positive	Positive by BF	Positive by CF	Positive by both tests
From surgically proved cases	18	18	7	7
From patients without accompanying clinical histories	190	164	43	17
Totals	208	182	50	24

2 and the titers of the individual sera in Table 3. While all 18 specimens from the proved cases were positive by BF with titers ranging from 1:10 to 1:2,560, only 7 were positive by CF with a titer range of 1:4 to 1:128. There was little correlation quantitatively. For example, the titers of serum E-1024 were 1:1,280 by BF and 1:16 by CF, while the titers of E-692 were 1:10 and 1:16, respectively. Of the remaining 190 serologically-positive sera, 164 were positive by BF, 43 by CF and only 17 positive by both tests. The majority of these sera had a flocculation titer of 1:5 (Table 3).

An unexpectedly low incidence of positive reactions (4% by BF, Table 4) was found in the wild-animal sera, since cross reactions with animal helminth infections were anticipated. The majority of the serologically positive sera came from bobcats, foxes, rabbits, and raccoons. The titers were consistently low.

To check possible cross reactions with antibodies to other antigens, sera from domestic and laboratory animals were titrated by BF before and after immunization with other antigens. Although 5.1 per cent of the immunized animals reacted with echinococcus antigen, 3 per cent of them also reacted before immunization. Goat, chicken and some horse sera gave very strong atypical reactions both before and after immunization and are not included in the table since no titers could be obtained. All other titers were low.

Although all the antigens prepared could be adsorbed onto bentonite and adjusted satisfactorily, there was great variation in the titers obtained with sample groups of sera, and comparable results could not be obtained. Table 5 shows the results of an early comparison of four

TABLE 3

Flocculation titers of human sera showing positive serological reactions

Source of sera	No. positive	Titers					
		Neg.	1:5	1:10	1:20	1:40	1:80+
From surgically proved cases	18	0	0	4	1	1	12
From patients without accompanying clinical histories but with positive serological tests	190	26*	142	18	4	0	0

* Positive by complement-fixation

TABLE 4

Reactions obtained by BF and CF tests on animal sera

Source of serum	Sera	Pos. BF		Pos. C-F	
		No.	%	No.	%
Wild animals. 12 species	1,742	70	4.0	8	0.4
Laboratory animals before immunization*	99	3	3.0	Not tested	
Laboratory animals after immunization	116	6	5.1	Not tested	
Totals	1,957	79	4.0	8	0.4

* Animals inoculated with various bacterial, viral and parasitic antigens, excluding cestodes

TABLE 5

270 human sera tested by the BF test with 4 antigens

Antigens	Positive	
	No.	%
Hog HF	26	9.6
Human HF	11	4.0
Horse HF	14	5.2
Type C scolices	113	41.9

antigens with 270 sera from Alaska. The titers ranged from 1:5 to 1:20 with the majority (92%) being 1:5 only. Only four of the sera were positive with all four antigens.

It should be noted that no quantitative chemical analyses were made, and the most efficient dilution for each antigen was determined empirically. It is probable that neither optimal nor comparable concentrations were used. Although there was great variation in the results, in general, it was noted that Type C scolices antigen gave the largest number of positive reactions, and human HF antigen the least. Type A scolices antigen appeared to react more specifically with the rabbit antisera as well as with the human diagnostic sera as evidenced by higher titers with proved-positive sera and fewer 1:5 reactions with probably-negative sera. However, the data are inadequate to draw definite conclusions about the antigens tested.

DISCUSSION

In the titration of 4,714 human and 1,957 animal serum samples collected in surveys of various populations in the United States and Alaska, the BF test showed a positive response in 4.6 per cent of the former and 4.0 per cent of the latter sera. The majority of the titers were 1:5. There are several possible explanations for these weak reactions. Some may be due to physicochemical changes in the serum which might be expected to influence the flocculation phenomenon. Two sera from a patient, diagnosed after surgery as having "infectious cirrhosis of the liver," which gave BF titers of 1:20 and 1:80, lend support to this theory. The possibility of cross reactions with antisera against closely related parasites has not been eliminated. The antigens are obviously crude, and some reactions may be between nonechinococcus antigens and antibodies. The level of the nonspecific reactions is low and further evaluation may indicate that 1:5 titers may be safely eliminated without false negative reports.

In the absence of complete clinical histories on the human sera, final evaluation of the BF reaction as a diagnostic test was impossible and no conclusions as to the specificity of the reactions could be drawn on comparative data when the tests were initially made. However, in every known instance where any echinococcus infection was verified by surgery, (we now have data on 35 verified cases) a positive BF reaction with a titer over 1:5 has been obtained. In one case, a serum sample sent several months after surgery was still positive with a lower titer (1:20 before and 1:10 after surgery) by BF but had become negative by CF. Such reactions suggest that the use of the BF in the diagnosis of human hydatid disease is justified as an experimental procedure in conjunction with the CF or other tests such as hemagglutination. Continuing studies with the BF and hemagglutination tests (Kagan *et al.*, 1959) have tended to substantiate this conclusion.

Without autopsy examinations on the wild animals, the significance of the serological reactions is not known. The relatively low percentage of positive reactions may be considered encouraging.

Of all the antigens prepared, the scolices antigens Type A and Type B show the greatest possibilities for further study. They may be more specific since they usually gave higher titers with proven-positive sera and fewer 1:5 reactions with the probably-negative sera. These antigens were somewhat more difficult to adjust on the bentonite, but once standardized, appeared to be as stable as the HF antigens.

SUMMARY

Comparison of results obtained by complement-fixation test and bentonite flocculation (BF) test on 4,148 sera from man and animals shows that the BF test gave more positive reactions and suggests that reactions below 1:10 titer may be nonspecific. Of a number of antigens tested on the bentonite particles, scolices antigens may prove to be more specific for use in the BF test than hydatid fluid antigen.

Although the BF procedure as a diagnostic test for human disease has been used with only 35 proven cases of echinococcus disease, it ap-

pears to merit consideration as another serological tool.

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AN EVALUATION OF THE HEMAGGLUTINATION AND FLOCCULATION TESTS IN THE DIAGNOSIS OF *ECHINOCOCCUS* DISEASE

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Echinococcosis of the liver, lungs and central nervous system has been shown to be associated with the presence of circulating antibody in the patient's serum. For this reason serological procedures can be used as an aid in the diagnosis of this disease. On the North American continent there are two species of *Echinococcus*. Both have potential public health significance since these species are believed to be infectious for man. *E. multilocularis* (Leuckart, 1863) has been reported from Alaska (Rausch and Schiller, 1951) and *E. granulosus* (Batsch, 1786) from Canada and the United States.

The serological diagnosis of echinococcosis has been based chiefly on results obtained by the Casoni skin test and the complement-fixation (CF) test. The sensitivity of the Casoni skin test varies from 53.8 per cent to 100 per cent (Meltzer *et al.*, 1956). As a diagnostic procedure for the detection of recent infection in endemic areas this test may be unreliable since, once acquired, skin sensitivity may persist for life. A positive test may, therefore, indicate only a previous exposure. The CF test has been reported to have a sensitivity of 52.4 per cent in proven cases by Meltzer *et al.*, (1956). Other workers have reported sensitivities varying up to 82 per cent. The limited sensitivity of the CF test indicates the need for more reliable serological methods in the diagnosis of this disease.

For the past several years we have been using the bentonite flocculation test, on an experimental basis, in addition to the CF test for the evaluation of echinococcus infection (Norman, *et al.*, unpublished). We have recently added the tannic-acid hemagglutination test for the diagnosis of this disease. Hemagglutination (HA) has been shown to be very sensitive in the detection of echinococcus antibody (Garabedian, Matossian and Djaman, 1957a, 1957b) and has proven to be effective in two other helminthic diseases (Kagan, 1955; Kagan and Bargai, 1956; Price and Weiner, 1956). The purpose of this report is to evaluate the sensitivity, specificity and reliability of the HA and bentonite flocculation (BF) tests in the diagnosis of echinococcosis.

MATERIALS AND METHODS

Some, but not all, sera were tested by the HA, BF, and CF methods. The following samples of human serum were used:

1. a. 30 sera from clinically diagnosed cases of echinococcosis, some of which were validated by surgery within 1 year of testing. In this group two sera from verified cases were received from Alaska, three from South America and ten from the continental United States.
   b. 8 lyophilized sera (0.25 ml) were sent from Lebanon.[1] These sera were reconstituted to 1 ml and used as undiluted specimens.
   c. 7 other sera were from cases in the United States for which we have no confirmatory surgical data.
2. 20 sera positive for trichinosis by BF test.
3. 20 sera positive for schistosomiasis by CF test.
4. 4 sera from proven cases of taeniasis (received from Lebanon).
5. 21 sera from dye-test-positive toxoplasmosis patients.
6. 19 sera from patients under observation for tuberculosis.
7. 20 sera positive for histoplasmosis by CF test.
8. 20 sera positive for syphilis.
9. 20 sera selected at random from Korean prisoners of war, collected in 1951.
10. 20 sera of residents in Washington, D. C.
11. 20 sera of residents in Atlanta, Georgia.
12. 34 sera of Arizona Indians from the San Carlos Reservation.
13. 24 sera of Alaskan Eskimos selected from

[1] We wish to express our gratitude to Dr. G. A. Garabedian, American University of Beirut, for supplying us with these sera.

areas in which echinococcus disease is endemic.

14. 41 sera submitted for routine diagnosis of echinococcosis. Since clinical histories were not available these specimens must be considered only as suspected cases of echinococcosis.

The antigen employed in all three tests was pig-hydatid fluid that was dialyzed for 24 hours in running water and concentrated 3-fold by per-evaporation. A nitrogen content of 10 mg per cent was obtained by micro-kjeldahl tests.

The tannic-acid HA test of Boyden (1951) was followed quite closely. Sheep erythrocytes in Alsever's solution or 3.8 per cent citrate were washed three times in phosphate-saline buffer (pH 7.2) and adjusted to a 2.5 per cent suspension with the buffered saline. An equal volume of 1:20,000 tannic acid in buffered saline was added and the mixture incubated at 37°C for 10 minutes. After washing the cells free of excess tannic acid with buffered saline, to each volume of cells a 1:16-dilution of hydatid-fluid antigen in buffer was added and the cells were incubated at room temperature for 15 minutes. A few ml of 1:250 normal rabbit serum in buffer were added to the cell-antigen mixture and centrifuged at 1,000 rpm for 5 minutes. The packed cells were resuspended in 1:250 normal rabbit serum in buffer as a 2 per cent suspension and used as antigen. Two-fold serial dilutions of sera were made with 1:100 normal rabbit serum in buffer starting at 1:50 (some sera were tested at 1:10) in tubes 10 × 70 mm. One drop of antigen-treated cells was added to 0.5 ml of diluted serum. The pattern of the erythrocytes on the bottom of the tube was read after 3 to 4 hours and after standing at room temperature overnight. The end point of the test was a clear mat of cells across the bottom of the tube equivalent to the 4-plus reaction figured by Stavitsky (1953).

The BF test was essentially the same as the BF test used in this laboratory for the diagnosis of trichinosis (Sadun and Norman, 1955). A suspension of bentonite particles 1 to 2 μ in size is mixed with antigen. The antigen used to coat the bentonite particles was the same 3-fold concentrated hydatid fluid used for erythrocytes in the HA test. On a wax-ringed slide 1 drop of bentonite-antigen suspension was added to 0.1 ml of serum and the slide was rotated for 15 minutes before being read. Sera diluted with saline 1:5, 1:10, 1:20 were screened for flocculation. Positive sera were titrated to an endpoint.

The 50 per cent CF tests (Schubert *et al.*, 1955) were performed in the serology laboratory of the Microbiology Section under the direction of Dr. K. W. Walls. We wish to acknowledge with thanks his permission to use his data for this evaluation.

TABLE 1

Sensitivity of three serological tests in detecting antibody for Echinococcus *disease*

Sera from	Sera	Positive hemagglutination		Positive flocculation		Positive complement-fixation	
		No.	%	No.	%	No.	%
Verified cases, North America	15	15	100	13	86.6	5	33.3
Verified cases, Lebanon	8	8	100	7	87.5		
Clinical cases	7	6	85.7	7	100	3	42.8
Totals	30	29	96.6	27	90.0	8	36.3

EXPERIMENTAL RESULTS

The serological findings in 30 clinical cases of echinococcus disease were divided into three groups: (verified cases from North America, verified cases from Lebanon and clinical cases from North America).

The HA test was positive for all but one serum (Table 1). The HA titers of sera from verified cases in North America ranged from 1:20 to 1:25,600; for Lebanon sera from 1:100 to 1:25,600; and for the sera from clinical cases from 1:80 to 1:6,400. Our highest titers were obtained with serum drawn from patients with viable cysts, or shortly after operation. Three sera from Alaskan patients (not listed in Table 1) from whom cysts had been removed 5 to 10 years earlier, were negative in all tests. One American patient, at the time of surgery had a HA titer of 1:3,200. One month after surgery the titer fell to 1:800.

Flocculation tests were performed on all of these sera. In 13 of 15 verified North American cases, titers of 1:5 to 1:2,560 were obtained. Negative tests were obtained with two sera that had low HA titers. Flocculation titers of 1:5 to 1:80-plus were obtained for seven of eight Lebanon sera. One Lebanon serum with a 1:100 HA titer was

TABLE 2

Serological reactions with sera from patients with other diseases when tested by hemagglutination and flocculation

Diagnosis of patient	Sera	Positive hemagglutination		Positive flocculation	
		No.	%	No.	%
Trichinosis	20	5	25	0	0
Schistosomiasis	20	5	25	0	0
Taenia infections	4	0	0	0	0
Toxoplasmosis	21	1	4.8	0	0
Tuberculosis	19	3	15.7	0	0
Histoplasmosis	20	0	0	0	0
Syphilis	20	0	0	0	0

TABLE 3

Serological reactions with normal sera when tested by hemagglutination and flocculation

Type of serum	Sera	Positive hemagglutination		Positive flocculation		Positive complement-fixation	
		No.	%	No.	%	No.	%
Normal sera from:							
Korea	20	1	4	0	0		
Washington, D. C.	20	1	4	0	0	0	0
Atlanta, Ga.	20	1	4	0	0		
Arizona Indians	20	8	32	20	100	0	0
Arizona Indians	18	4	22.2	0	0	0	0
Eskimos	24	6	25	5	20.8	0	0

negative by BF test. Flocculation titers of 1:20 to 1:1,280 were recorded for the seven sera from clinical cases in the continental United States. In one case a titer of 1:160 was obtained with a serum that was negative by HA test.

The CF test was positive with 5 of the 15 verified North American cases of echinococcus disease. The Lebanon sera were not subjected to the CF test in our laboratory. Three of seven in the clinical group were positive with titers of 1:4 to 1:16.

The specificity of the HA and BF tests was evaluated for 124 sera from patients clinically ill with protozoan, helminthic, treponemal and mycotic diseases (Table 2). The HA test showed some nonspecific activity with trichinosis, schistosomiasis and tuberculosis sera. The titers obtained with the trichinosis sera ranged from 1:50 (3 sera) to 1:100 (2 sera). In no instance was the erythrocyte pattern on the bottom of the tube typical of the pattern produced by positive echinococcosis sera, but all varied sufficiently from the controls to be listed as questionable positives. The three positive tuberculosis sera had titers of 1:50, and the 5 schistosomiasis sera titers of 1:100 to 1:800. A number of schistosomiasis sera showed some activity in the first tube (1:50) but were not listed as positive because on retesting they were negative. After standing for 48 hours all of the positive schistosomiasis sera became negative. Positive control sera, after 48 hours, tend to hemolyze; whereas, negative sera continue to produce the typically negative cell-pattern. Flocculation tests with all these sera were negative.

A number of sera collected from areas that might be termed nonendemic (Washington, Atlanta and Korea), from an endemic region (Alaska), and from a rural area in the United States where the population is engaged primarily in cattle raising (Arizona), were tested for HA and BF activity with echinococcus antigen (Table 3). Three of 60 sera from the nonendemic areas gave positive HA tests. Two sera had a titer of 1:50, one serum from the Atlanta sample a titer of 1:100.

The sera from Indians of Arizona were divided into two groups on the basis of BF titers obtained in an earlier survey (Norman *et al.*, unpublished). One group comprised 20 sera selected from a sample of 194 which had been found positive by BF test (1:5), the second group included 18 sera which had given negative BF tests. Eight sera from the positive-flocculation sample were positive by HA tests with titers ranging from 1:50 to 1:400. The erythrocyte patterns in these tests were atypical since the cells agglutinated in the form of "ripples" which did not resemble the smooth mat of cells produced by positive echinococcus sera. Four of the 18 negative sera gave positive HA tests with titers ranging from 1:50 to 1:300.

Six sera from Alaskan Eskimos gave positive HA tests, ranging in titer from 1:100 (4 sera) to 1:200 (2 sera). Flocculation titers of 1:5 to 1:20 were obtained with five sera. With only four sera were positive titers obtained by both HA and BF tests. In essence therefore, seven sera of the 24 tested (29%) gave positive serological reactions. The CF tests on the sera tested in each group were negative.

A total of 41 routine diagnostic sera from patients with suspected cases of echinococcosis were tested by the HA method. Six (14.6%) had low titers (1:100) in the HA test. Two sera (5%) gave positive BF reactions (1:5) and all gave negative CF tests. To date, of 411 such routine diagnostic sera tested in our laboratory 40 have been found positive by BF test (9.9%) and 20 by CF test (4.8%).

A positive human serum from a patient with an acute echinococcus infection was used as a control in 16 tests over a 3-month period. The HA titer of this serum was 1:6,400 in six tests and varied one or two tubes, plus or minus, in all of the other tests. A 1-tube variation, plus or minus, was obtained in five tests, a 2-tube variation in four tests and a 3-tube variation in one test. The overall average titer for the 16 tests was 7.74 tubes (8 tubes = 1:6,400). In addition to the positive control serum, one of two normal human sera were tested in parallel with the positive human echinococcus serum. In five of the 16 tests a titer of 1:100 was obtained with the normal human serum. This activity was subsequently found to reside in one of the two normal sera and this normal serum control was subsequently replaced.

DISCUSSION

The HA tests described here appear to be slightly more sensitive than the BF or the CF test in the detection of echinococcus antibody in confirmed or clinically diagnosed human sera. In testing nonechinococcus sera a certain degree of cross activity was observed (14.2% of 242 sera). It should be noted however, that had we wished, we could have eliminated most of these low-titer results since the agglutination of the sheep cells by them was not characteristic of the pattern produced by positive control sera. Moreover, on standing, some of these low-titer sera became negative, as was noted for the schistosome sera. We did not eliminate these anomalous results because these reactions indicate a certain degree of nonspecific activity in the HA test. This atypical agglutination pattern must be recognized in any laboratory that will use the procedure. With experience, atypical HA patterns are readily recognized. Titers of 1:50 to 1:100 in HA tests must be evaluated very carefully since in five of sixteen instances one of our normal control serums had titers of 1:100. Perhaps with the use of antigens other than hydatid fluid these low, false-positive titers can be eliminated. In the presence of clinically active cases the HA titers are very high and the agglutination of the sheep cells very strong.

The BF test was slightly less sensitive than the HA test in detecting active infection. However, the BF test did not give as many false-positive reactions. Negative tests were observed with nonechinococcus sera in all but the Arizona Indian and Eskimo serum samples. In previous studies (Norman *et al.*, unpublished) eight of 145 sera from patients ill with other diseases had given positive flocculation tests (5.1%). For the purpose of calculating the percentage of false-positive reactions in this evaluation the results of BF tests obtained with the Arizona Indian sera should be excluded since 20 sera giving positive flocculation tests from a sample of 194 were specifically selected to evaluate the HA test. In the original study (Norman *et al.*, unpublished), 18 per cent (35 sera of 194) were positive by flocculation. With the Arizona Indian sera excluded, less than 2 per cent false-positive reactions by the BF test were recorded in this evaluation.

The data are not available but it would have been of interest to know the extent of parasitosis in these Indians showing the false positive reactions. It would have been interesting to know if the sera giving positive reactions were from individuals infected with related cestode infections. With four sera from patients infected with *Taenia saginata* the HA test was negative. One might expect some cross reactions with other cestode species. Sera from rabbits injected with *Taenia taeniaformis* antigen cross-reacted very strongly with echinococcus-sensitized cells in the HA test.

The percentage of positive HA (25%) and BF (20.8%) tests with Eskimos sera agree quite closely with the numbers of skin-sensitive Indians (31%) in Northwest Canada (Wolfgang and Poole, 1956). It should be re-emphasized however, that the HA titers with these sera were low and the erythrocyte patterns atypical. In each instance a question mark was placed after the titer, indicating uncertainty at the time they were read.

With proven echinococcus human sera the HA test has been very consistent and stable with various lots of sheep cells. The data from this

paper and the data from Garabedian *et al.*, (1957a, 1957b) indicate that this serological procedure, in spite of its high sensitivity, may be a very useful diagnostic tool in the detection of active cases of echinococcus infection. The use of dialyzed, concentrated, hydatid fluid as an antigen in the HA test has certain disadvantages because of false patterns observed with serum diluted 1:50 to 1:100. Early in the work sera were titrated from 1:10 and a high incidence of titers to 1:40 were obtained with nonechinococcus sera. To eliminate these nonspecific reactions sera were titrated from 1:50. Had we titrated our sera from a dilution of 1:100 many of the false tests would have been eliminated without seriously affecting the sensitivity of the test.

The CF test with pig or human hydatid antigen appeared to lack sensitivity in the detection of echinococcus antibody. Attempts to increase the sensitivity of this test result in anti-complementary serum. Perhaps with the development of other antigens the CF test can be made more sensitive.

On the basis of the limited data so far obtained in our evaluation studies a serum, submitted for diagnosis of echninococcosis, which has a HA titer of three tubes or greater (1:200+) and a positive BF test of 1:5 or greater may be considered to be presumptive evidence of active infection with this disease. A serum with a low HA titer (1:50 or 1:100) and a negative BF titer, or the reverse, a low BF titer and a negative HA titer, may reflect nonspecific serological responses. Until a greater number of proven positive sera have been made available for testing with both the HA and BF tests, it is advisable that all diagnostic sera be tested with both these procedures.

SUMMARY

Boyden's tannic acid hemagglutination test (HA) and a bentonite flocculation test (BF) were evaluated for the detection of echinococcus disease in sera from 30 clinically diagnosed patients. In addition, the sensitivity of the test was evaluated with 246 sera from nonechinococcus individuals.

The HA test was slightly more sensitive (96.6% of 30 sera) than the BF test (90.0% of 30 sera) in the detection of positive clinical echinococcus infections. In the titration of 246 nonechinococcus sera 14.2 per cent gave positive results with the HA test (titers of 1:50 or 1:100 in most instances). With the BF test 10.1 per cent of these sera were positive. The CF test was positive in only 36.3 per cent of 22 sera from patients with active infections of echinococcosis but showed no cross reactions with the nonechinococcus sera tested.

A positive HA test and a positive BF test given by a serum from a case clinically diagnosed as echinococcosus, constitute presumptive evidence for the presence of echinococcus disease.

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PROBABLE BIOLOGICAL CONTROL OF SCHISTOSOMIASIS MANSONI IN A PUERTO RICAN WATERSHED

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The status of schistosomiasis has been studied intermittently in the Los Peña community of the Quebrada San Antón watershed, east of Rio Piedras, Puerto Rico. The suburban valley of Los Peña has an area of 10.3 square miles and has 19 tributaries of the main stream Quebrada San Antón as well as others adjacent. The terrain consists of rolling hills which gradually become an alluvial plain before ending at the Laguna San José. The principal stream is normally not more than 10 feet wide, and only occasional pools are more than 6 feet deep (Fig. 1). All tributaries are narrow and shallow. The first studies, conducted by Pons and Hoffman (1933), comprised clinical observations on acute and chronic cases, and determinations of population density and infection rates in the snail intermediate host of *Schistosoma mansoni*, *Australorbis glabratus*.

In 1947, the Los Peña area was used as a test area in which all available methods of schistosomiasis control were utilized (Oliver-González *et al.*, 1950). Treatment with stibophen and other antimonials was administered, water supply was improved, a latrine was installed at every home, and health education was greatly stressed. This study covered a period of 6 years from 1947 to 1953. The data in Table 1 show that the prevalence of *S. mansoni* dropped from 44.6 per cent in 1947 to 4.5 per cent 2 years later. This case rate decline is an example of the efficacy of stressed health education, therapy, and other control measures, but it is also an example of the temporary effect of such measures, since the infection rate had increased to 29.2 per cent by 1953 when the control program ceased.

In 1950 sodium pentachlorophenate was tested in a section of the main stream adjacent to the housing area (Berry *et al.*, 1950). The chemical was very effective in killing *A. glabratus* in the area treated and for about 1.5 miles downstream, but snails returned 6 to 8 months later.

In 1952 the large, tropical, South American ampullarid *Marisa cornuarietis* was first seen in the Los Peña section of the stream (Fig. 2) (Oliver-González *et al.*, 1956). By March, 1956, this snail had advanced upstream for about 1 mile to a small dam only 18 inches high, which temporarily stopped further migration. A gradual decrease in *Australorbis* has been observed in all stream sections where *Marisa* has become established. However, infected *Australorbis* continued to be found in these reduced colonies during the interim 1952 to 1956, from the main stream or tributaries, ranging up to 8 per cent positive. During 1956, infected *Australorbis* were known to be in only one tributary in the stream system. No infected snails have been found since 1956. This finding was based on the first snail survey of the entire watershed stream system completed March, 1956, and repeated bimonthly since then. *Australorbis* was found to be absent from the 1.5-mile-long section of the main stream having the greatest numbers of *Marisa*, which section includes the Los Peña community. However, the two species were observed living in average numbers (5 to 20 per square yard) in some tributaries.

Watershed populations of *Australorbis* continued to decline as *Marisa* increased up to October, 1957, when only small numbers of the host species persisted at three places. The effect of *Marisa* on *Australorbis* has been studied in the laboratory by Chernin *et al.*, (1956), who found that the former snail is a voracious feeder on vegetation and that it ate *Australorbis* eggs and reduced numbers of young snails. The number of snails per volume of water was shown to be an important factor.

Beginning in March, 1956, *Marisa* has been planted in lots of 200 large specimens at all upstream and downstream sections, as well as paralotic swamps and isolated oxbows. Replantings were made as necessary through October, 1957. Further observations will show whether the entire stream and tributaries will remain populated with *Marisa* and, in particular,

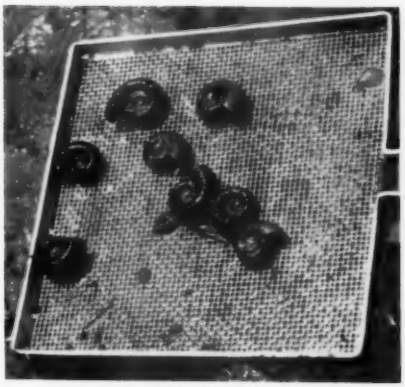

FIG. 1. Mature *Marisa cornuarietis*, competitor of *Australorbis glabratus* in Puerto Rico.

whether *Australorbis* will disappear in the three tributaries where both species are still together; the host species being present at less than 1 per square yard.

The prevalence of *Schistosoma mansoni* in humans during 1956 and 1957 was 6.0 and 7.7 per cent, respectively (Table 1). Sampling of the same families for all surveys listed has always been done on a house-to-house basis in the crowded areas bordering the stream. It is doubtful if new cases are now appearing at Los Peña. This is suggested by the data presented in Table 2, which summarizes prevalence during 1956 and 1957 in the sensitive 0-to-10-year age groups from the study area, as well as in other similar watersheds in which control has not been done. In the 1956 and 1957 sampling, an effort was made to make the majority of tests among preschool and school age persons, who are most apt to contact watershed streams at play or enroute to schoo'. Of 922 persons in Los Peña examined in 1957, 7.7 per cent were positive, but the number of infected individuals of the 0-to-16-year age group did not increase over 1956. The youngest infected individual was 5 years old. This individual probably became infected during the time when *Australorbis* were still numerous in 1952. The negativity of all persons sampled up to 5 years of age suggests that the watershed is now free of infective *Schistosoma mansoni*.

Examinations in similar watersheds where there has been no control and where no *Marisa* has been planted are presented in Table 2. The results of examinations in these areas differ markedly from those in Los Peña, indicating that some factor was present in the latter to reduce infection of the human host.

TABLE 1

Summary of schistosomiasis surveys by fecal testing in Los Peña community, Río Piedras, Puerto Rico, from 1947 through 1957

Date	Tested*	Pos. (%)
Jan. 1947†	500	44.6
Sept. 1948†	467	8.9
Aug. 1949†	779	4.5
July 1953‡	500	29.2‖
Apr. 1956§	746	6.0
July 1957§	922	7.7

* Single stools from individuals were examined using the $ZnSO_4$-Triton-ether centrifugation method.

† Oliver-González *et al.*, 1950.

‡ Data furnished by courtesy of Dr. José F. Maldonado, University of Puerto Rico, School of Medicine-School of Tropical Medicine.

§ Commonwealth of Puerto Rico, Department of Health (Bilharzia Control Unit) and U. S. Public Health Service Puerto Rico Field Station survey data.

‖ Increase in prevalence between 1949 and 1953 may have been due to relapse among treated cases.

Details of the field biology of *Marisa* (Ferguson and Oliver-González, 1958) and its growing use as a control factor in reducing *Australorbis* populations in special Puerto Rican habitats have been described. *Marisa* becomes most numerous (as medium and large sizes) in the quieter stretches of Quebrada San Antón lined with *Caladium*, on which it feeds. Few egg masses are seen in this creek, which is subject to periodic washouts, particularly during April to November. In the tributaries, great numbers of small *Marisa* are commonly seen foraging ceaselessly. It is thought that the combination of interference with normal *Australorbis* colonization in the protected tributaries, and competition with the stronger snail, *Marisa*, in the storm-affected main stream, results in decimation of the host species. *Marisa* has not yet reached its population peak as determined by the carrying capacity with respect to the abundant food supply in the watercourses and swamps of the valley.

To a moderate extent *Marisa* was adversely affected by pollution at three places in the valley:

FIG. 2. View of Quebrada San Antón, Los Peña community, Río Piedras, Puerto Rico

TABLE 2

Comparison of schistosomiasis prevalence during 1956 and 1957 in the 0–10-year age groups of Los Peña and other endemic basins where control has not been done

Annual surveys	Los Peña	Caguas (Bunker)	Caguas (Checo)	Naguabo (Daguao)
1956	0% (0/239)	2.0% (2/99)	11.7% (18/153)	8.7% (12/137)
1957	1.8% (6/329)	2.4% (1/41)	9.6% (10/104)	20.3% (35/172)

below the effluent point of a small abatoir for about 500 yards, in an oxbow serving as a dump area for slum housing, and below the effluent pipe of a modern sewage disposal system (activated sludge type) for about 0.25 mile. However, *Marisa* has operated effectively against *Australorbis*, even in these marginal environments. This predator species does not accept infection with *S. mansoni* under laboratory testing conditions.

SUMMARY

The Los Peña community, now emerging from a suburban slum status to a stabilized, small, housing settlement, has been the object of various schistosomiasis studies since about 1933. Trials at stopping transmission among the dense creekside population have included stibophen chemotherapy, health education, improved domestic water supplies, installation of sanitary privies, and a single chemical molluscicide test. Although those controls were partially effective, the disease remained significantly endemic through 1952. Since this time, surveys of the snail intermediate host species, *Australorbis glabratus*, in the main stream showed it to be in precipitous population decline, faced with a phenomenally large colony of an ampullarid snail, *Marisa cornuarietis*, first seen in the stream in 1952. During March, 1956, this highly competitive species was successfully transplanted to all upstream stations in the watershed, and during a period of 1.5 years has almost eliminated the host species in these areas.

Two recent successive annual surveys of the same creekside families indicate that no new infections are appearing in the statistically sensitive preschool age group. The community preva-

lence in all ages and both sexes has levelled off at a rate of about 6 or 7 per cent. This first instance of probable biological control of schistosomiasis transmission should be repeated in other endemic environments capable of supporting the remarkable snail *Marisa*. This field test is a step toward the solution of certain control problems in Puerto Rican schistosomiasis on a permanent and economically feasible basis.

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PERSISTENT EXCRETION OF PYRIMETHAMINE FOLLOWING ORAL ADMINISTRATION[1]

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Previous studies in this laboratory (Smith and Ihrig, 1957) on the metabolism of pyrimethamine (2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine) in two human subjects indicated that this compound was excreted rather slowly following single oral doses. These findings appeared to explain the protracted protection afforded by the compound against subsequent infection of human subjects with *Plasmodium vivax* (Coatney *et al.*, 1953). Interest in the mass use of widely-spaced doses of pyrimethamine in suppressing malaria in endemic areas made it desirable to define in more precise terms the pattern of urinary excretion following varying doses of this drug and, in particular, the length of time during which effective suppressive levels of the compound would be maintained. Studies designed to provide this information were undertaken with the results summarized in this report.

MATERIALS AND METHODS

General. Serum levels and urinary excretion of pyrimethamine[2] after oral administration were investigated in ten healthy human volunteers, each receiving a single 100-mg dose. Sterile, clotted blood samples were obtained at 1, 3, 6, and 10 days after dosing, and 24-hour urine specimens were collected at 20, 30, 52, and 62 days after drug administration. Urine samples were acidified to pH 4 with dilute HCl as collected, and stored at 6°C. An aliquot of each 24-hour collection was sterilized by filtration through sintered glass; the remainder was stored at −20° C.

The same ten subjects were used in subsequent studies in which single, oral, 0.25-mg and 1.0-mg doses of pyrimethamine were given. Renal excretion of pyrimethamine was determined for a 24-hour period following each dose.

Analytical procedures. The concentrations of pyrimethamine in serum and urine samples were determined usually by microbiological assay using *Streptococcus faecalis* as the test organism. In some instances these findings were confirmed by chemical determinations of pyrimethamine using the spectrophotometric method of Schmidt *et al.*, (1953). Details of both procedures are given by Smith and Ihrig (1957).

RESULTS

Concentrations of pyrimethamine in serum. Following single oral doses of 100 mg, levels of pyrimethamine in serum fell slowly from an initial average value of 0.57 mg/L 24 hours after dosing to an average of 0.12 mg/L nine days later (Table 1). It was not practical to determine serum levels beyond the tenth day. However, it was found that by plotting the logarithm of the serum concentration on Days 1, 3, 6, and 10 against time (arithmetic scale), fairly straight lines were obtained. Thus, it was possible to predict by extrapolation the anticipated serum level at any time interval after dosing. Included in Table 1 are the extrapolated serum levels which one would expect to be present in each subject 30 days after a single, oral, 100-mg dose of pyrimethamine.

Renal elimination of pyrimethamine. Additional information concerning the persistence of pyrimethamine in these human subjects was obtained by analyzing 24-hour urine samples collected at 20, 30, 52, and 62 days after drug administration. These data, shown in Table 2, indicate that all subjects continued to excrete pyrimethamine in the urine for at least 30 days after a single oral dose of 100 mg. Excretion on Day 20 averaged 120 ± 17 μg; on Day 30, 35 ± 9 μg. No pyrimethamine was detected in any of the urine samples obtained on the 52nd and 62nd days after dosing. Hence, the subjects stopped excreting pyrimethamine in detectable quantities sometime between the 30th and the 52nd days after treatment.

[1] This study was supported in part by research grant E-824 from the National Microbiological Institute, National Institutes of Health, U. S. Public Health Service.

[2] We are indebted to Dr. George Hitchings, Wellcome Research Laboratories, Tuckahoe, N. Y., for the pyrimethamine used in these studies.

TABLE 1

Concentration of pyrimethamine in the serum of human subjects following a single oral dose of 100 mg

Subject	Serum level in mg/L* after days				
	1	3	6	10	30†
JP	0.60	0.46	0.25	0.17	.019
RS	0.78	0.47	0.27	0.17	.016
AG	0.60	0.42	0.26	0.16	.013
LS	0.58	0.37	0.25	0.12	.005
GB	0.47	0.35	0.22	0.11	.005
RH	0.52	0.28	0.17	0.09	.004
TJ	0.52	0.40	0.21	0.12	.004
ES	0.49	0.30	0.17	0.08	.0025
CS	0.61	0.42	0.22	0.10	.002
MA	0.56	0.37	0.19	0.09	.0015
Mean	0.57	0.38	0.22	0.12	.007

* Measured by microbiological method
† Estimated by extrapolation (See text)

TABLE 2

Pyrimethamine content of human urine samples collected over 24-hour periods at varying times after dosing

Dose of pyrimethamine (*mg*)	Time after treatment (*days*)	Excretion of pyrimethamine μg per 24 hours* (*Mean* ± *std. error*)
100	20	120 ± 17.1
	30	35 ± 9.1
	52	0
	62	0
0.25	0†	16 ± 3.3
1.0	0†	29 ± 3.0

* Measured by microbiological method
† Urine collected for 24 hours after dosing

Although one cannot determine directly the fraction of the original dose of pyrimethamine remaining in a human subject at a given tme after dosing, the problem can be approached indirectly by comparing the amount of pyrimethamine excreted in a given 24-hour period with the amount which is excreted during the initial 24-hour period following the administration of a single small dose of pyrimethamine. Thus, when these subjects were given a single oral dose of 0.25 mg, they excreted 16 ± 2 μg during a 24-hour period; following a 1.0-mg dose they excreted an average of 29 ± 3 μg. This latter figure agrees fairly well with the urinary excretion observed during the 24-hour period 30 days after administration of a 100-mg dose of pyrimethamine. From this it was concluded that human subjects retain, on the average, about 1 mg (1 %) of a 100-mg dose of pyrimethamine at the end of 30 days.

TABLE 3

Summary of the urinary excretory patterns of pyrimethamine in ten human subjects following a single oral dose of 100 mg

Days after dosing	Per cent of dose excreted in urine
0–5	8–16
6–11	4–8
12–16	2–4
17–25	1–2
26–40	0.5–1
Beyond 40	negligible
Total for 40 days	16–32

DISCUSSION

By combining the results of this study with those obtained previously by Smith and Ihrig (1957), it is possible to describe the overall pattern of urinary excretion of pyrimethamine in human subjects (Table 3). These findings indicate that urinary excretion of pyrimethamine is a slow, persistent process and accounts for 20 to 30 per cent of the pyrimethamine administered. Urinary excretion beyond the 40th day is negligible.

These data also provide a basis for estimating the time interval after a single, large, oral dose of pyrimethamine during which one would expect human subjects to be protected against infection with *P. vivax*. Thus, Hernandez *et al.*, (1953) demonstrated that daily doses of pyrimethamine of the order of 0.25 mg were sufficient to protect most human volunteers against infections with *P. vivax*. Our data indicate that human subjects will retain amounts of pyrimethamine in excess of this amount (0.25 mg) for periods of about 30 days. One would expect some to be protected for less than 30 days, while others in a group might be protected for as long as 40 days. One would scarcely expect any protection beyond the 52nd day since by this time all subjects in this study had ceased excreting detectable quantities of pyrimethamine in the urine.

It should be pointed out that these data are not presented in substantiation of the use of large, widely-spaced doses of pyrimethamine in suppressing malaria. Although Miller (1955) apparently obtained a great reduction in parasite rate through the monthly administration of 25-mg doses of pyrimethamine to groups of natives living in Liberia where *falciparum* and *ovale* malaria are endemic, it seems obvious from other published investigations (Jones, 1954; Grounds, 1954; Clyde, 1954; Clyde and Shute, 1954, 1957; Schneider *et al.*, 1957) that this method of treatment would provide an ideal opportunity for the emergence of pyrimethamine-resistant forms.

SUMMARY

The concentrations of pyrimethamine in the serum, and excretion of this drug in the urine, were studied in ten healthy, human subjects following administration of single oral doses of 100 mg. The average concentration in serum fell slowly from 0.57 mg/L 24 hours after dosing to 0.12 mg/L nine days later. Urinary excretion continued for at least 30 days, but the compound was no longer detectable in the urine 52 days after single, large, oral doses. These data, correlated with observations on urinary excretion of pyrimethamine following administration of minimal suppressive doses of the drug, suggest that single 100-mg doses of pyrimethamine should protect most humans from infection with sensitive strains of malaria parasites for about 30 days. However, it is pointed out that such widely-spaced doses might well provide an excellent opportunity for the emergence of pyrimethamine-resistant organisms.

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INTESTINAL PROTOZOANS AND HELMINTHS IN AMERICANS RESIDING IN SOUTHERN TAIWAN (FORMOSA)[1]

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Among peoples who live and travel abroad, whether by choice or in fulfillment of military and diplomatic duties, etc., there is a certain fear of infection by pathogens which do not occur at "home." In some situations there may be cause for concern but in a great many others real fear for extraordinary infections is not warranted. In the case of parasite diseases, extensive travel and temporary residence in foreign areas do not necessarily imply high risks of infection, especially if one uses judgment in his eating and drinking habits and practices the standard rules of sanitation by which the majority of westerners live. However, extended residence, *i.e.*, months or several years, in areas where parasite infection potentials are high, may account for alterations in, or additions to, an individual's intestinal fauna.

The present study was undertaken to evaluate the hypotheses given above and to determine the incidence of intestinal fauna among Americans stationed in the southern part of the island of Taiwan. Since most of the subjects available for study had lived on Taiwan for more than 1 year it was believed that an examination of stool specimens, especially from military dependents, would indicate whether residence in a new environment had noticeably altered the parasite picture in comparison with that expected of a similar group of Americans living in the United States.

MATERIALS AND METHODS

The stool specimens upon which this study is based were obtained from American military personnel and their dependents living in or near the southwestern coastal cities of Kao-hsiung and Tso-ying. Most of the stools from military personnel orignated from males who submitted fecal samples for their annual physical examinations. Samples from dependents were obtained upon a request basis as well as from persons calling at the Navy dispensary for treatment of routine ailments or for examination.

Contributors were provided with a one-page questionnaire requesting general information on their eating habits, *i.e.*, whether they ate at local restaurants, sources from which fresh foods were procured, general occurrence of diarrhea, etc. Although a few children are represented in the survey, the majority of persons were adults in the general age range of 24 to 45 years.

In order to facilitate proper and quick handling a given number of persons was instructed to submit specimens on an appointed day. Fecal samples were brought to the dispensary in sputum cups, the majority within 2 to 4 hours after passage. Several samples the size of a pea were taken from different parts of each stool, well-macerated with an applicator stick and fixed in MIF (Merthiolate-iodine-Formalin) solution in 15-ml screw-cap vials. Three direct-smear preparations (22-x-22-mm cover slip) were made from a small quantity of material pipetted from the upper layer of sediment. Although the direct smear allows study of only a small sample there is several-fold concentration of protozoans and helminth eggs by the sedimentation principle, especially if vialed specimens are allowed to stand until the supernatant preservative is clear.

SURVEY FINDINGS AND DISCUSSION

Table 1 lists the species and incidence of intestinal protozoans and helminth eggs detected in an examination of 300 American military personnel and dependents residing in the southern part of Taiwan. Since the majority of specimens originated from adults living in a rather restricted area, no attempt has been made to categorize individuals by their specific service or military occupation, by specific areas of residence or by unusual activities. However, since the occupational duties of active military personnel introduce factors for possible influence on parasitism, the dependents are listed separately. The over-all number of specimens is

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of the Navy.

TABLE 1

Incidence (per cent) of intestinal protozoans and helminths in Americans living on Taiwan*

Intestinal fauna	Military	Dependents
Protozoa		
Entamoeba histolytica (LR† only)	4	1
Entamoeba histolytica (SR‡ only)	2	1
E. histolytica (LR & SR together)	1	1
Total *E. histolytica*	6	2
Entamoeba coli	7	4
Endolimax nana	6	3
Iodamoeba bütschlii	—	—
Dientamoeba fragilis	4	3
Giardia lamblia	7	3
Chilomastix mesnili	1	—
Trichomonas hominis	1	—
Enteromonas hominis	4	1
Helminths		
Ancylostoma	1	1
Ascaris lumbricoides	17	10
Enterobius vermicularis	4	1
Heterodera sp.	1	—
Trichuris trichiura	4	3
Specimens with no protozoa	85	87
Specimens with no helminths	81	87
Specimens with neither protozoans or helminths	74	78
Total number of fecal specimens examined	116	184

* Given as nearest whole number.

† LR = large-race *E. histolytica* with cysts measuring more than 10$_\mu$.

‡ SR = cysts measuring less than 10$_\mu$.

not great but represents a good sample of the American personnel residing in this particular area.

There are many variables in the reporting of the intestinal fauna of man. Consequently, there is no satisfactory means for critical comparison of data obtained from peoples studied by various investigators in one area with those from another geographic region. However, it is apparent, as judged by our figures, that the Americans concerned in the present report are not subjected to living conditions which greatly influence the over-all variety and incidence of their intestinal protozoans and helminths, *if* we use the incidences generally reported for the United States as a standard for comparison. The species represented are essentially those expected to be found if a similar survey were made in this group residing in the United States. Percentages of incidence, however, are slightly higher than those (Kuntz, unpublished data) recorded for Navy personnel passing through the hospital at the National Naval Medical Center, Bethesda, Maryland.

With the exception of the figures given for *Ascaris*, the incidences for the different intestinal fauna are not great and fall considerably below those listed by Faust and Russell (1957) for the United States. This is especially true of *Entamoeba histolytica*, the parasite of greatest concern. Faust and Russell, in listing the results of surveys of different workers over a period of years, show many infection rates in excess of 20 per cent.

Although the total incidence for *E. histolytica* is low, this parasite is much more prevalent in the strictly military personnel than in their dependents, *i.e.*, 6 and 2 per cent, respectively. The greater incidence for the large race *E. histolytica* in the military column is a point of some interest since as a rule the nonpathogenic, small race of *E. histolytica* is the predominant form. We are unable to account for the absence of *Iodamoeba bütschlii* since it normally occurs in lists given in studies made in temperate as well as tropical zones. On the other hand, the incidence of *Dientamoeba fragilis* is comparatively high and speaks well for the method of stool preparation employed. It is believed that the over-all low incidence of protozoans may be a reflection of the rather wide use of antibiotics among service personnel. The fact that several individuals harbored *E. histolytica*, *E. coli*, *E. nana* and *D. fragilis* suggests that they probably had not been exposed to antibiotics for some time prior to collection of study materials.

Actually, the higher rates for all protozoans and helminths, and *Entamoeba coli* in particular, among military personnel suggest that they are subjected to conditions which are more favorable for host-to-host transfer of intestinal fauna than are their dependents. This is to be expected since the duties of the former group take them into areas where circumstances tend to encourage parasite acquisition and the chances for intestinal fauna carriers are increased several fold. The figures for infection by *Ascaris*, are

none too surprising since this parasite is common on Taiwan (a conservative estimate of over-all incidence of *Ascaris* has been estimated at 60 to 70 per cent) and the chances for contamination of hands and food are great. In spite of the incidence of *Ascaris*, 74 per cent of the military personnel and 78 per cent of their dependents had no parasites or other fauna.

The living habits of the American families are not comparable to those experienced by troops living abroad in a combat or training status, but our data sheets indicate that dependents do live in such a manner as to make infection by parasites possible if they are lax in public health practices and careless in their eating habits. Approximately 48 per cent of families obtained fresh food from local markets; 76 per cent employed cooks, amahs or houseboys who handled food at some stage in preparation for consumption; and 63 per cent of military personnel and their dependents visit and eat in local restaurants or in the homes of local acquaintances. Questioning revealed that most people eating local foods select only those which are steaming hot. Fortunately, most of the Chinese foods are characteristically well cooked and served so hot that even a contaminated plate is sterilized.

The majority of military personnel eat at one time or another in hostels and clubs where local food handlers are employed. In some instances these, as well as employees in private homes, could serve as possible sources for infection. However, rather strict foodhandler regulations assure diners of reasonably safe food. A recent examination of servants in the homes of Americans in the Taipei area revealed an unusually low level of parasitism. As a matter of fact the number of negative stools was a point of some concern in our examinations. It must be emphasized, however, that persons seeking employment in American homes and establishments are aware of the westerners' demand for cleanliness and of the fact that certain physical requirements must be met. Questioning has revealed that foodhandlers, especially, follow a course of self treatment with antibiotics or other drugs prior to submission of stool specimens for examination.

Participants in this survey admitted that they had experienced diarrheas more frequently than in the United States. Six per cent reported frequent diarrheas with accompanying intestinal involvements. Clinical records and stool examinations, however, revealed no amebae and suggested that these conditions were due primarily to bacterial infections. The majority of persons included in this survey had resided on Taiwan for 14 to 24 months and 19 per cent of the dependents had made visits or business trips beyond the limits of the island.

Much has been written regarding the acquisition of intestinal parasites by veterans during World War II and the subsequent public-health hazards. Emphasis has been given particularly to the influence of foreign duty and residence upon the incidence of amebiasis. Lincicome, *et al.*, (1950) found that there was a definite increase in *E. histolytica* in service veterans who had been overseas as compared with those who had not. The same study revealed, however, that the incidence of *E. histolytica* in postwar student populations composed of men with military service was no greater than before the war and these authors concluded that there was little change in the general incidence of amebiasis as a result of war time activities. Kean and Smillie (1954) made comparisons of intestinal protozoans in American travelers before and after visits to Europe. They came to the conclusion that there was no major increase of *E. histolytica* and other intestinal protozoans. Although Jacobs, *et al.*, (1946) examined numerous personnel at separation camps and found a marked difference between *E. histolytica* infection in troops who had served in different areas outside the United States, and in those who had not, the general concensus of opinion has been that the experiences of war and travel have exerted little influence on the general incidence of amebiasis.

In most studies amebiasis has been the object of most concern, with little attention directed to the nonpathogenic intestinal fauna, some of which serve as excellent indicators, reflecting carelessness in personal hygiene and in eating habits.

SUMMARY

Our study indicates, as have others, that it is difficult to make evaluation of the influence of military duties and foreign residence upon the intestinal fauna of a given group of people since there are no suitable criteria or standards for comparison. Nonetheless our figures based upon an examination of a single stool specimen

from each of 300 Americans indicate that military duties for the individual concerned in this study have exerted more influence on the incidence of intestinal fauna than has residence of their dependents in more restricted areas of Taiwan. With the exception of *Ascaris*, which occurred in 17 and 10 per cent, respectively, of the military personnel and their dependents, the over-all incidence of intestinal fauna is slightly greater than that recorded for a group of Navy personnel and dependents studied in the United States. The incidence of intestinal protozoans was low, due possibly to the common use of antibiotics by service personnel, and the large race was the predominant form of *Entamoeba histolytica*.

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A CORRELATIVE STUDY OF IMMUNOLOGICAL TESTS FOR THE DIAGNOSIS OF HYDATID DISEASE

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Immunological procedures employed for the diagnosis of hydatid disease do not seem to be properly standardized. Controversial reports by various investigators concerning complement-fixation (CF), precipitation and intradermal (ID) tests have created much confusion in the literature dealing with hydatid disease. Such discrepancies have resulted from the utilization of a wide variety of antigens and methods. For example, as source of antigen some workers have used crude hydatid fluid (HF), filtered or nonfiltered, others have employed "purified" fluid or extracts of scolices, and still others have resorted to non-specific sources, utilizing extracts of other helminths which presumably have common antigenic factors with hydatid scolices. Likewise, the methods employed by different investigators have varied tremendously. Careful titration of the antigens used in CF tests have been carried out by only a few workers. The dose of antigen injected for skin tests, the time for taking the readings and the manner of interpretation of results have been constantly variable. The lack of consideration of optimum antigen-antibody ratios in precipitation tests has made the results of this test unreliable. The existence of common antigenic components among various cestodes, sensitivity of certain individuals to some non-specific proteins present in the antigens used for skin tests and miscellaneous other factors have also contributed in obtaining false positive results.

The recent application in this laboratory of an indirect hemagglutination (IHA) test for hydatid diseasd (Garabedian *et al.*, 1957a) has created a new interest in the serology of this disease. The adsorption on tanned erythrocytes of a sensitizing antigen from HF results in an antigen complex which subsequently reacts with serum antibodies in high titers. Preliminary experiments indicated that this test yields a high percentage of positive results with serums obtained from hydatid disease patients, and gave no false positive results in individuals not suffering from hydatid disease.

In view of these findings further studies were carried out on various groups of individuals to correlate and compare the results obtained in IHA, CF and ID tests. The data accumulated from such studies are given in this communication. The uniformity of the procedures employed in this study, and careful standardization and preservation of the antigen used for these determinations, have added to the reliability of the results. The information obtained from post-operative findings has further facilitated the interpretation of the results.

MATERIALS AND METHODS

Serum samples. Samples of blood were obtained from 184 patients admitted to the Medicine and Surgery Wards of the Hospitals of the American University of Beirut. Of these patients 79 were diagnosed clinically and confirmed by surgical intervention as having hydatid disease of liver, lung or other organs and areas. The remaining 105 patients suffered from other illnesses. In the latter category of patients diagnoses were established either by surgical intervention or by clinical and laboratory studies. These patients were not suspected of having hydatid disease. Blood samples were collected prior to testing of patients with Casoni skin-test material. In each case the serum was separated from the clot and stored at −20°C until use. For tests each sample was thawed and inactivated at 60°C for 20 minutes.

Antigen. Collection, titration and storage of HF antigen for CF and IHA tests are described elsewhere (Garabedian *et al.*, 1957a). Fluid was aspirated from hydatid cysts of cattle and collected in separate flasks. Only those fluids were considered satisfactory which were relatively clear, not anti-complementary, and contained many scolices. The titration of the antigen was carried out either by a modification of Kolmer's method described elsewhere (Garabedian *et al.*,

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1957a, b), or by the procedure of IHA tests. In this latter procedure various concentrations of the fluid were used for sensitizing the tannic-acid-treated erythrocytes. The coated erythrocytes were subsequently tested for their agglutinating ability. The hydatid fluid was considered as a good antigen for these tests when, by the CF method of antigen titration, it showed a titer of 1:2 or 1:4 (the optimum dilution of antigen that gives the most sensitive reaction with the smallest amount of serum), and/or by the technique of IHA gave a titer of 1:2 (the highest dilution of antigen that sensitizes the erythrocytes to give a most sensitive reaction with the smallest amount of serum). A known positive serum, collected from a patient with hydatidosis, was used for both of these determinations. Various samples of cyst fluids showing satisfactory titers were pooled in one flask. This constituted the stock antigen. This was stored either at −20°C in sealed ampoules, each containing 5 ml, or lyophilized in appropriate amounts and kept at 4°C. For CF and hemagglutination tests the necessary amounts were removed, thawed or rehydated to the original volume.

For skin tests, the stock antigen was filtered through a Seitz filter and phenol was added in a concentration of 0.5 per cent. Sterile phenolyzed saline (0.5 per cent) was used as control. The test fluid was stored in the cold at 4°C and was used within 6 months.

Complement fixation test. The procedure employed for these tests has been reported in a previous communication (Garabedian *et al.*, 1957a). Doubling dilutions of each serum to be tested were prepared in 0.1-ml amounts and to each serum dilution was added an equal amount (0.1 ml) of a 1:4 dilution of hydatid fluid. The mixtures were allowed to stand at room temperature for about 15 minutes after which 0.2 ml of complement, containing 2 full units, was added to each tube; the tubes were left overnight at 5°C. The following day 0.2 ml of sensitized sheep erythrocytes was added to each of the tubes, the tubes were placed in a water bath at 37°C and the readings were taken after 30 minutes. The titer of the serum was the highest dilution which showed less than 25 per cent hemolysis.

Intradermeal tests. Filtered and phenolyzed hydatid fluid, 0.2 ml., was injected intradermally into the flexor surface of the forearm, while a similar amount of phenolized saline was injected in a like manner into the second forearm. The reactions were read after 20 minutes. In positive cases a wheal formation with pseudopodia and surrounding redness occurred, while the control injection showed no such reaction.

Indirect hemagglutination test. This test was performed by treating human O, Rh-negative erythrocytes with a 1:20,000 dilution of tannic acid (Merck) for 10 minutes in a water bath at 37°C, and subsequently coating them with a 1:2 dilution of hydatid fluid. This was done by allowing the erythrocyte-hydatid-fluid mixture to stand for 25 minutes at room temperature. To 0.25 ml of 2-fold dilutions of inactivated serum, an equal amount of 0.5 per cent sensitized erythrocytes was added and the tubes were allowed to stand at room temperature for 2 hours. In positive tests, the first few tubes showed heavy agglutinating masses of erythrocytes, an effect which, depending on the titer of the serum, gradually disappeared in higher dilutions. In negative tests, the erythrocytes settled at the bottom of the tubes and attained a button-like appearance.

RESULTS

Table 1 compares the result of CF, ID and IHA tests on 184 patients with hydatid disease and/or other illnesses. Of 30 patients with hydatid disease of the liver, 26 gave positive results in CF tests, 26 in ID tests and 27 in IHA tests; the percentages being: 86.7, 86.7, and 90.0, respectively. Of the 30 patients with hydatid disease of the lung 21 gave positive results in CF tests, 27 in ID tests and 23 in IHA tests; the percentages being: 70.0, 90.0 and 76.7, respectively. Of 19 patients with hydatid disease of miscellaneous organs or areas, 14 gave positive results in CF tests, 17 in ID tests and 19 in IHA tests; the percentages being: 73.7, 89.5 and 100, respectively. The mean percentages for the total of 79 cases tested by CF, ID, and IHA tests were 77.2, 88.6, and 87.3, respectively.

Of the 105 patients suffering from illnesses other than hydatid disease six gave positive results in CF, 19 in ID tests and 0 in IHA tests; the percentages being 5.9, 18.1 and 0, respectively. Three of the serums from 105 patients tested by CF gave anticomplementary results.

Table 2 gives the titer ranges in CF and IHA tests of serum samples obtained from patients with hydatid disease and other illnesses. It can be seen that of a total of 79 patients with hydatid disease of liver, lung and miscellaneous organs

TABLE 1

Cumulative results of complement fixation, intradermal and indirect hemagglutination tests on patients with hydatid disease and other illnesses

	Cases	CF			ID		IHA	
		Pos.	%	ac*	Pos.	%	Pos.	%
Hydatid disease of:								
liver	30	26	86.7	0	26	86.7	27	90.0
lung	30	21	70.0	0	27	90.0	23	76.7
misc. areas†	19	14	73.7	0	17	89.5	19	100
mean			77.2			88.6		87.3
Other disease‡	105	6	5.9§	3	19	18.1	0	0

* Anticomplementary.

† Kidney, brain, heart, spine, abdomen, eye and thigh.

‡ Includes cases of hypertension, rheumatic fever, peptic ulcer, pyelonephritis; cancer of stomach, brain, orbit, lung and liver; hepatomegaly, hepatosplenomegaly, ascites, cirrhosis of liver; taeniasis, ascariasis, amebiasis, and miscellaneous other conditions.

§ Calculated after deducting 3 anticomplementary serums from the total number of 105.

TABLE 2

Titer ranges of complement fixation and indirect hemagglutination tests in patients with hydatid disease and other illnesses

	Reciprocal of dilution of serum					
	CF			IHA		
	2–8	16–64	128–512	10–160	320–5120	10,240–163,840
Hydatid disease of:						
liver	8	12	6	1	11	15
lung	6	12	3	2	15	6
misc. areas	3	8	3	2	11	6
Totals	17	32	12	5	37	27
Other disease	3	3	0	0	0	0

or areas, in CF tests, 17 had a titer range of 1:2 to 1:8; 32, 1:16 to 1:64; 12, 1:128 to 1:512. In IHA tests five had a titer range of 1:10 to 1:160; 37, 1:320 to 1:5120; 27, 1:10, 240 to 1:163,840.

Of the 105 serum samples obtained from patients suffering from illnesses other than hydatid disease, in CF tests three had a titer range of 1:2 to 1:8, 3, 1:16 to 1:64 and none, 1:128 to 1:512.

TABLE 3

Correlative data on patients with hydatid disease showing positive reactors in complement fixation, intradermal and indirect hemagglutination tests

Positive reactors among 79 cases of hydatid disease	CF	ID	IHA	%
57	+	+	+	72.15
6	−	+	+	7.59
5	−	+	−	6.33
4	−	−	+	5.06
3	−	−	−	3.79
2	+	−	+	2.53
2	+	+	−	2.53

On the basis of these findings it may be stated that the IHA test as applied for the diagnosis of hydatid disease is a more specific test than are the CF and ID tests, and yields much higher titers than the CF test.

Correlative data on patients with hydatid disease showing positive reactions to the CF, ID and IHA tests are presented in Table 3. Of the 79 patients, 57 (72.15%) were positive reactors to all three tests. The remaining patients reacted only to one or two of these tests.

DISCUSSION

The literature dealing with the immunological aspects of hydatid disease is extensive. Antigens and methods used for making CF, ID and precipitation tests have varied much in the hands of different investigators and the results obtained have been controversial in many instances (N. H. Fairley, 1922; K. D. Fairley, 1923; Hiles, 1927; Dennis, 1937; Godfrey, 1937; Senekji, 1941; Culbertson *et al.*, 1941; Graña, 1946; Belding, 1952, and Bensted *et al.*, 1953). Thus, in some instances skin tests have been found to be more sensitive than the CF tests while in other instances the reverse has been reported.

Bensted *et al.*, (1953) indicated the importance of utilization of standardized reagents for the serologic reaction of hydatid disease. By careful titration of hydatid-fluid antigen with a positive serum they found that 93 per cent of the proved hydatid cases gave positive results in CF tests and 86 per cent in ID tests. The titration of the

hydatid fluid prior to its use in CF tests seems to be important. In the experience of the authors an appreciable number of positive serums were missed by the use of diluted antigens. Conversely, a large number of nonspecific reactions were obtained when hydatid fluid was used in an undiluted form. Although antigenic potency of various samples of hydatid fluid may vary, in most instances fluids rich in scolices usually show a titer of 1:4 when titrated with known positive serums.

The use of purified material extracted from hydatid fluid has given satisfactory results in the hands of some investigators (Dennis, 1937, Senekji, 1941). In our opinion such material should not be used in immunological tests until it is demonstrated conclusively that the extracted subetances contain the whole, active antigen against which the antibodies are directed. These substances may consist of proteins, polypeptides, polysaccharides or may be complex antigens hitherto undetermined (Čmelik, 1952). A trichloroacetic acid-precipitated portion of hydatid fluid (Dennis, 1937) was tested in this laboratory by IHA, and was found to have no advantage over the crude fluid in sensitizing the erythrocytes. In our experience the most satisfactory method of preserving the antigen has been either to store the hydatid fluid at −20°C or to lyophilize it. This latter method can be used for sending the powdered antigen to countries where fresh hydatid fluid is not available.

Hydatid fluid filtered through Seitz filter failed to sensitize tanned erythrocytes. This was possibly due to the retention by the asbestos pad of the sensitizing substance during the process of filtration. Such fluids showed a marked decrease in protein content, but their polysaccharide content remained unaltered. Fluids filtered in this way gave variable results in CF tests but could be used successfully in ID tests.

The existence of antigenic overlapping among various groups of cestodes has led some investigators to prepare antigens from related worms for use as skin test material in hydatid disease (Culbertson *et al.*, 1941). It should be stated, however, that serum samples obtained from 15 individuals harboring adult *Taenia* spp. when tested in this laboratory with hydatid fluid antigen, failed to reveal antibodies to this antigen either by IHA or CF tests. The results of ID tests with hydatid antigen were also negative in these cases.

The absence of false positive reactions in IHA tests may be due to a phenomenon of specific adsorption of the reactive protein on the tanned erythrocytes. As in most serologic tests false negative reactions may be obtained on serums of patients who fail to develop circulating antibodies. Such results may be due to the inability of the antigen to get through the cyst wall, to the poor antibody response of the host to the inciting antigen, or to infected, calcified, or sterile cysts which fail to supply adequate antigenic stimuli to the host tissues (Hiles, 1927). For example, when tested in this laboratory, of the ten patients with hydatid disease who gave false negative results in IHA tests, seven had infected, calcified or sterile cysts with no scolices. These findings were confirmed by postoperative pathological examinations.

SUMMARY

A correlative study of the three immunological tests, complement fixation, indirect hemagglutination and intradermal tests was carried out on 79 patients with hydatid disease and 105 patients with miscellaneous conditions. The mean percentages of positive reactors among the 79 patients with hydatid disease tested by complement fixation, intradermal and indirect hemagglutination tests were, respectively, 77.2, 88.6 and 87.3. Among the 105 patients with conditions other than hydatid disease the percentages of positive results were 5.9 in complement fixation, 18.1 in intradermal and 0 in indirect hemagglutination tests. On the basis of these findings it may be stated that indirect hemagglutination as applied for the diagnosis of hydatid disease is a more specific test than are complement fixation and intradermal tests.

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NOTES ON PARACHOLERA IN SULAWESI (CELEBES)

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El Tor vibrios were first differentiated by Gotschlich (1906) who isolated them at the El Tor Quarantine Station on the Sinai Peninsula from pilgrims returning from the Hejaz. These organisms were cultured from persons with and without diarrhea, and from corpses of individuals who had died of other causes during the pilgrimage. El Tor vibrios were found repeatedly in mild diarrheas and in symptomless persons. They were also seen in Bengal and Madras, where they were isolated from wells and from carriers without clinical disease (de Moor, 1949). El Tor vibrios, which seem to cause no disease or only mild symptoms in other parts of the world, nevertheless produce severe, cholera-like disease in Sulawesi (de Moor, 1938, 1939, 1939a, 1948, 1949; van Loghem and de Moor, 1940). The malady in Sulawesi has been called paracholera El Tor and enteritis choleriformis El Tor van Loghem (de Moor, 1948, 1949). El Tor disease differs from cholera in that the causative agent of El Tor disease is pathogenic for man only occasionally and in a geographically circumscribed area; by its static character; by the sporadic occurrence of cases; and by the low morbidity in afflicted towns and villages (de Moor 1948, 1949).

Sulawesi was free from paracholera for 13 years. In 1957, however, a fresh outbreak occurred. The purpose of this communication is to discuss the last two epidemics, which have not as yet been described. The present writer had the good fortune to partake in the investigation of all four of the known outbreaks of paracholera in Sulawesi.

THE CAUSATIVE ORGANISM

For the isolation of vibrios, spontaneously evacuated stools, rectal swabs and vomitus are examined.

If transportation of the specimens to the laboratory is necessary, a tube containing 5 ml of 1 per cent alkaline-peptone water is inoculated with a swab, then the contents of this tube are streaked to diagnostic plates. Dieudonné's classic alkaline-blood-agar plate, and alkaline agar have been used for primary inoculation. The latter is prepared by adding 10 ml of a 1N $Na_2CO_3 \cdot 2H_2O$ solution to 300 ml of 3 per cent nutrient agar.

Plates and tubes are incubated for 8 hours at 37° C. Then one loopful from the surface of the peptone water is streaked to Dieudonné and alkaline-agar plates. The growth on the alkaline agar after 8 hours incubation is abundant enough to permit the use of the microscopic agglutination test with high-titer *Vibrio comma* 0 sera, and to inoculate differential tubes for pure culture studies. The growth on the Dieudonné plates is much slower. The alkaline-agar plates permit a preliminary diagnosis within 10 hours after the specimens reach the laboratory, especially since frank cases often yield abundant pure cultures.

Sucrose, mannose and arabinose have been used in the primary differentiating biochemical series, since El Tor vibrios belong to the first group of Heiberg, as do cholera strains. Members of this group form acid but no gas from sucrose and mannose, leaving arabinose unchanged. All vibrios isolated in Sulawesi belonged to Heiberg I group, biochemically and serologically.

Differential agglutination in alkaline medium (Tanamal, 1948) is also employed. In this test, dilutions of high-titer Inaba and Ogawa sera without any preservative are made up in 0.3 per cent $Na_2CO_3 \cdot 2H_2O$. Two-fold serial dilutions are prepared, putting 1-ml aliquots of the dilutions into serologic tubes. A few drops of a thick suspension of the vibrios in distilled water are added to each tube and the mixtures incubated at 37° C. Cholera vibrios do not show agglutination or are agglutinated only in low serum dilutions (not higher than 1:100), while El Tor strains show flocculation in tubes to the end-titer of the serum. The organisms isolated from the outbreaks in Sulawesi followed the pattern of El Tor vibrios.

Since the hemolysin production of individual, freshly-isolated, vibrio cultures may differ greatly, as shown by Otten (1939) and de Moor (1939), not much reliance was based on this test. The proof of endotoxin production also varies with the strains. Moreover, laboratory animals in great numbers are required for toxin determina-

tion. Thus, these tests were not carried out in routine, practical, diagnostic work.

El Tor vibrios are supposed to produce acetylmethyl-carbinol, *i.e.*, to give a positive Voges-Proskauer test. Using the Barritt technic, some strains isolated in Sulawesi varied in their acetylmethyl-carbinol production. As a matter of fact, the very first El Tor strain seen in this laboratory, was initially Voges-Proskauer negative.

According to Gipsen (1938), cholera vibrios become inagglutinable when heated at 56° C for 3 hours, while El Tor strains retain their agglutinability. Meyer (1939) described a test according to which cholera vibrios become inagglutinable when suspended in 2 per cent chloroform, while El Tor vibrios do not. There are, however, too many exceptions in both tests. Linton (1940), showed that there are different protein fractions in cholera vibrios and in El Tor strains. This method is too laborious to be carried out in a diagnostic laboratory.

All El Tor strains formed indol and gave a positive cholera-red test.

As far as the susceptibility of the El Tor strains to antibiotics and sulfonamides is concerned, Table 1 shows that Synthomycetin, Chloramphenicol and streptomycin checked their growth in dilutions as low as 1 μg/ml of broth.

TABLE 1

Average drug susceptibility of El Tor strains in vitro after 24 hours' incubation at 37° C

Drug	Growth in concentration (μg/ml) alkaline-peptone water						
	0.1	0.5	1	5	10	50	100
Synthomycetin	+	+	0	0	0	0	0
Chloramphenicol	+	+	0	0	0	0	0
Streptomycin	+	+	0	0	0	0	0
Tetracycline	+	+	+	0	0	0	0
Chlortetracycline	+	+	+	+	0	0	0
Oxytetracycline	+	+	+	+	0	0	0
Sulfapyridine	+	+	+	+	+	+	0
Sulfaguanidine	+	+	+	+	+	+	+
Sulfadiazine	+	+	+	+	+	+	+
Sulfanilamide	+	+	+	+	+	+	+
Sulfacetamide	+	+	+	+	+	+	+
Sulfamethazine	+	+	+	+	+	+	+
Percoccide	+	+	+	+	+	+	+

EPIDEMIOLOGY

El Tor disease, called paracholera, has been observed in successive epidemics in several areas of the southwestern peninsula of Sulawesi. Between periods of occurrence, the disease is not seen on the island for several years. To date, four epidemics have been noted. The first two occurred before World War II.

A. On the west coast of Sulawesi. This epidemic lasted for 8 months, from September, 1937, to April, 1938. A coastal area, about 100 km long, north and south of Makassar, and somewhat inland, was afflicted, together with islands in the Spermonde Archipelago off the coast of Sulawesi. In all, 48 cases were registered, of which 26 appeared during the dry season from September until November, involving Pangkadjene, Barroe and the island of Samatello. Then 22 cases were noted from January to April during the wet season, of which two were in Makassar, five in Limbung, seven north from Barroe, five on the islands of Salemo and Sanane, and three south of Pangkadjene.

B. Eastern areas and east coast. Sixty-two persons became ill. During 7 months, from October, 1939, to April, 1940, 37 patients were reported in the area around the Tempe Lake. In addition, 15 cases developed in December, 1939, in localities about 40 km northeast from the Tempe Lake. In June and July, 1939, 10 cases were seen about 120 km south from the lake area.

The first two episodes of El Tor paracholera in Sulawesi were summarized by de Moor (1948, 1949). His findings were: average case mortality 70 per cent; average morbidity 4 per 1,000 inhabitants of infected villages; 10 per cent of family members excrete vibrios; most cases occur sporadically; casual carrier rate 4 per 1,000; El Tor vibrios found in 43 per cent of the wells, also in rivers where cases occurred; and in 4 per cent of all examined wells. Vibrios are seldom found in water, however, after the disease passed in the respective locality.

The third El Tor outbreak occurred during World War II.

C. West and east coast. Seven persons were involved in 1944. One case occurred in Makassar during February, one in Takalar in March, and five in Watampone during the months of June, September and December.

This epidemic took place during the Japanese occupation. The manager of the Grand Hotel in Makassar, a Japanese, became suddenly ill with vomiting and diarrhea in the middle of February,

1944, and died within 12 hours after the first symptoms set in. The vomitus and the stools were streaked to Dieudonné plates, from which pure cultures of vibrios were isolated. These were agglutinated by cholera 0 antiserum. The sublimate-precipitation and the alkaline agglutination tests confirmed the isolates as El Tor vibrios. This writer considered the organisms El Tor vibrios but the Japanese-occupation authorities believed that the illness was classic cholera. The staff and the guests of the hotel were strictly isolated. Cholera vaccine was prepared locally and administered on a large scale. A search for carriers was initiated. Of the 300 hotel guests, four yielded El Tor vibrios. Several hundred other contacts did not harbor this organism at the time of the examination. Of the several wells examined in Makassar only one, located about 4 km from the hotel, harbored El Tor vibrios. There was no paracholera among the users of that well, or in its surroundings. As a matter of fact, the Japanese hotel manager was the only case observed in the town of Makassar during this outbreak.

About 3 weeks later, in early March, 1944, an Indonesian inmate of the Takalar prison, which is about 50 km from Makassar, was found dying with frequent vomiting and profuse diarrhea. El Tor vibrious were isolated from the rectal swab. Water samples from the wells and the fish ponds surrounding the prison were not found to be infected but El Tor vibrios were cultured from the river running behind the prison. An investigation of the prison inmates yielded six carriers.

No further cases were reported until June. Since the Japanese occupation authorities carried out the epidemiologic studies, no particulars could be obtained except a statement that five cases and six carriers were diagnosed in Watampone during June, September and December, 1944. The 11 cultures which were turned over by the Japanese laboratory to this institute and which were said to be isolated from these persons, proved to be El Tor vibrios.

After this episode, no paracholera was observed for 13 years in Sulawesi.

Then the fourth (and present) epidemic broke out.

D. In the capital Makassar. Seventy cases were diagnosed between January, 1957, and February, 1958. There were 52 cases between 17 January and 18 April, 1957, then 12 from 2 June to 15 July, followed by single cases on 30 August, 18 September, 7 October, 9 November, 15 January and 8 February. At the same time, 81 persons ill with paracholera were reported in adjacent areas: four in Djeneponto in February 1957, three in Sengkang in April, 44 in April–May in Pare-Pare, five in Sindjai in May, ten in Watampone and 15 in Madjene; a total of 151 cases in Sulawesi in 1 year.

Two cases were seen in Djakarta on the island of Java. No new occurrences were reported after February, 1958.

Of the 70 patients observed in Makassar, 31 were taken to hospitals. Thirteen of them died, having arrived too late for treatment, or succumbed to intercurrent diseases after having been free from vibrios. The remaining 18 persons recovered. Thirty-nine individuals did not ask for medical help and died in the villages. Their corpses, however, could be examined bacteriologically, thanks to the autopsy decree which now makes such investigations possible.

Most cases, 41, occurred on separate days. Five were reported on the same day in one instance, four in one instance, three in four instances and four in 4 instances. Thus, dispersion over a long period, as well as sporadic infections, were characteristic of this epidemic.

Table 2 shows the distribution of the patients by age and sex. Most of them were between 16 and 50 years of age. With the exception of the group over 50 years, the number of sick males and females appeared to be about equal.

Epidemiologic investigation revealed that on 21 and 27 January, respectively, a mother and one of her daughters died suddenly after having suffered from vomiting and diarrhea. These cases are not listed as reported. Two other daughters, living in the same house, died of paracholera on 29 January and 1 February, respectively. These two are found in Table 3 which represents all patients from whom, either during the disease or

TABLE 2

Distribution according to age and sex of patients during the 1957–1958 outbreak in Makassar

Age group	Female	%	Male	%	Together	%
Over 50	3	4.3	9	12.9	12	17.2
16–49	21	30.0	21	30.0	42	60.0
10–15	4	5.7	3	4.3	7	10.0
3–9	4	5.7	5	7.1	9	12.8
Totals	32	45.7	38	54.3	70	100.0

TABLE 3*

Bacteriologically confirmed instances of paracholera in Makassar during the 1945–1958 epidemic

No.	Age	Sex	Duration of disease before admission	Hospitalized or DOA		Symptoms	Treatment	Recovered or died	Note
				Hospital	Date				
1	35	m	?	LBH	17/1/47	D, V, Sh	I, P, St, Tripyron	R 2/2	
2	3	f	?	SMH	29/1	D, V, Cy	Te	+29/1	22/1 & 27/1 sister & mother died—no bact. exam.
3	60	f	10 hrs	DOA	30/1	D, V, Cy			1 household member carrier
4	4	m	10 hrs	DOA	31/1	D, De, Cy, Ri			
5	56	m	?	RSU	31/1	D, V, Cy	Sg, I, Op	+1/2	
6	18	m	18 hrs	RSU	31/1	D, V, Cy	Sg, Op, Emetine	+31/1	
7	70	m	12 hrs	DOA	1/2	D, V			
8	76	f	6 days	DOA	1/2	D, V, Cy			
9	12	f	2 days	DOA	1/2	D, V, Cy			Sister of Case # 2
10	20	f	20 hrs	DOA	1/2	D, V, Cy			
11	16	f	16 hrs	DOA	2/2	D, V, Cy			
12	15	m	?	RSU	2/2	D, V, Cy	I, Sg, Ch	R 9/2	
13	30	m	?	RSU	2/2	D, V, Cy	I, Sg, Ch	R 5/2	
14	16	f	10 hrs	DOA	3/2	D, V, Cy			
15	30	f	?	RSU	3/2	D, V, Cy, (He)	I, Ch	+ 6/2	
16	76	f	18 hrs	DOA	3/2	D, V, Cy, Ri			
17	57	m	1 day	DOA	3/2	D, V, Cy			
18	9	f	14 hrs	DOA	3/2	D, V, Cy			
19	52	f	?	RSU	4/2	D, V, Cy	DBT	+ 4/2	
20	35	m	1 day	DOA	4/2	D, V, Cy			
21	50	f	1 day	DOA	4/2	D, V, Cy			
22	25	f	?	LBH	5/2	D, V, Sh	I, St, Ch, Sg	R 16/2	
23	33	f	?	RSU	5/2	D, V, Cy	I, Ch	R 15/2	
24	54	f	1 day	DOA	5/2	D, V, Cy			
25	17	f	10 hrs	DOA	7/2	D, V, Cy			
26	72	f	18 hrs	DOA	8/2	D, V, Cy			
27	6	m	10 hrs	DOA	8/2	D, V, Cy			
28	40	m	?	LBH	11/2	D, V, De	I, St, Sd, Ch	R 23/2	
29	30	m	?	LBH	12/2	D, V, Cy	I, St, Sd, Ch	R 18/2	
30	45	m	16 hrs	DOA	13/2	D, V, Cy			
31	35	m	14 hrs	DOA	14/2	D, V, Cy			
32	3	f	10 hrs	DOA	18/2	D, V, Cy			
33	21	f	?	LBH	18/2	D, V	I, St, Ch, Sdi	R 28/2	
34	50	f	?	DOA	20/2	D, V, Cy			
35	60	f	?	LBH	21/2	D	Sdi, St, Ch	R 6/3	
36	15	f	1 day	RSU	21/2	D, V	I, Sg, Emetine, Yatren	R 2/3	
37	40	f	18 hrs	DOA	24/2	D, V, Cy			

TABLE 3—(*Continued*)

No.	Age	Sex	Duration of disease before admission	Hospitalised or DOA		Symptoms	Treatment	Recovered or died	Note
				Hospital	Date				
38	26	f	1 day	DOA	27/2	D, V, Cy			
39	30	m	2 days	SMH	8/3	D, V, (Pn)	I, St, P	+11/3	
40	35	m	?	SMH	8/3	D, V, Ri, Cy, Sp, De	I, St	+14/3	
41	20	m	18 hrs	DOA	10/3	D, V, Cy			
42	4	m	10 hrs	DOA	11/3	D, V, Cy			
43	8	f	14 hrs	DOA	17/3	D, V, Cy			
44	13	m	18 hrs	DOA	21/3	D, V, Cy			same house as #43
45	8	f	?	SMH	26/3	D	Sto	R 2/4	
46	39	m	20 hrs	DOA	27/3	D, V, Cy			
47	40	m	?	RSU	3/4	D, V, Cy, De (Ne)	I, Ch, Sg	+13/4	
48	16	f	16 hrs	DOA	9/4	D, V, Cy			
49	7	m	?	LBH	12/4	D, V, Sh, Pn	I, St, Ch	+12/4	
50	35	f	?	RSU	15/4	D, V, Cy	I, Ch	R 23/4	
51	36	f	1 day	DOA	16/4	D, V, Cy			
52	36	m	2 days	DOA	18/4	D, V, Cy			
53	15	m	1 day	DOA	2/6	D, V, Cy, De			
54	25	f	1 day	DOA	7/6	D, V, Cy			
55	10	m	?	SMH	8/6	D	Sto, Te, Ch	R 18/6	
56	30	m	?	SMH	9/6	D, V, Cy	I, St, Ch	R 18/6	
57	25	m	?	SMH	13/6	D, V, Cy	I, St, Ch	R	
58	25	f	?	RSU	16/6	D	Sg, Ch	R	
59	3	f	1 day	DOA	19/6	D, V, Cy, De			
60	40	f	?	DOA	27/6	D, V, Cy, De			
61	40	m	?	RSU	29/6	D, V, Ri, Cy, De	DBT	+29/6	
62	30	m	1 day	DOA	1/7	D, V, Cy			
63	40	f	20 hrs	DOA	2/7	D, V, Cy			
64	30	m	?	RSU	15/7	D, V, Ri, Cy, De	I, Sg, Ch	R	
65	24	m	?	DOA	30/8	D, V, Cy			
66	25	f	?	RSU	18/9	D	Sg, Ch	R	
67	31	m	1 day	DOA	7/10	D, V, Cy			
68	35	f	3 days	LBH	9/11	D, V, De	DBT	+9/11	
69	39	f	1 day	DOA	15/1/58	D, V, Cy, De			
70	30	f	?	RSU	8/2/58	D, V	I, Sg, Ch	R	

* DOA—dead on arrival, or not seen while alive but cultures taken from dead body; LBH—Labuang Badji Hospital; RSU—Public State Hospital; SMH—Stella Maris Hospital.

Cy—cyanosis; D—diarrhea; De—dehydration; (He)—heart failure during hospitalization; (Ne)—nephritis during hospitalization; Pn—pneumonia on admission; (Pn)—pneumonia during hospitalization; Ri—rice-water stools; Sh—shock; Sp—spasms; V—vomiting.

Ch—Chloroamphenicol; DBT—died before treatment; I—infusion of alkaline saline; Op—opium; P—Penicillin; Sd—sulfadiazine, orally; Sdi—sulfadiazine, intravenously; Sg—sulfaguanidine; St—streptomycin, parenterally; Sto—streptomycin orally; Te—oxytetracycline.

R—recovered.

post mortem, El Tor vibrios were isolated. A discussion of the treatment and the course of the disease is not within the scope of this report.

The total number of known cases in Makassar can be assumed to be 72. Since the registered number of inhabitants of the city was 326,748, the morbidity rate was about 2.2 per 10,000 inhabitants. The mortality rate of the patients was

high, about 90 per cent. This is in accordance with observations made during previous outbreaks.

In the Makassar area, the pattern of spread followed that considered typical for paracholera. The village populations are dense. The infection jumped from one village to another, eventually bridging a span of several km, without the mechanism of the transfer becoming apparent to us. There was one instance in which four deaths occurred in the same house, and two in which two inhabitants of the same dwelling became ill within the period of 1 week. In all other instances there was only one paracholera patient in each afflicted house. In only two families could all members of the household be examined. In the first, of ten apparently paracholera-free persons, there was one carrier. In the other, of 12 household members, two carried El Tor vibrios. Of the wells examined in three villages, two harbored these organisms. The same vibrio was isolated in the town from a ditch which ran beside a house in which paracholera had occurred. The household members claimed, however, that they had been using only water from the waterworks for consumption and utilities.

A search for symptomless carriers and for unreported cases was undertaken. Rectal swabs were collected from the corpses in all instances in which two or more persons died from any disease on the same day in the same locality. The swabs were inoculated into alkaline-peptone broth, and delivered to this laboratory. More than 300 corpses were examined but no vibrios were cultured. Neither were El Tor organisms isolated from the more than 1,000 stools from noncholeraic patients which were received in this laboratory for other than vibrio studies during 1957.

Epidemiologic investigations in localities distant from Makassar are difficult for technical reasons. Thus, when paracholera appeared in Djeneponto on the southern tip of the peninsula in early February, 1957, five clinical cases were reported with three deaths, but no bacteriologic specimens could be transported to the laboratory.

In other outlying localities, in April, 1957, three clinically diagnosed cases appeared in Sangkang, east from Tempe Lake. All three died. At the same time, an outbreak started in Pare-Pare, north from Makassar, from which 44 patients with 40 fatalities were reported. Only one stool specimen was received which was found to harbor El Tor vibrios.

In mid-May the disease disappeared from Pare-Pare but spread north to Madjen where 15 cases with 11 deaths were registered. This town had not been infected during previous epidemics. Also, only one stool specimen could be sent to Makassar from Madjen. It was found to contain El Tor vibrios. Then Sindjai, east of Makassar on the Gulf of Borneo, reported five cases with three fatalities, and Watampone, north of Sindjai, reported ten cases, all of which died.

Thus, 81 persons were diagnosed clinically as paracholera patients outside the Makassar area, of whom 71 died, a lethality of about 88 per cent. Due to difficult communications, water and contacts would not be examined.

Because of the occurrence only of sporadic cases over the past months, from August, 1957, to February, 1958, one may believe that paracholera is assuming an endemic character in Sulawesi.

DISCUSSION

A disease caused by El Tor vibrios has been observed in Sulawesi in four epidemics, the last of them seemingly taking on an endemic character. The illness is serious. A high per cent of the cases observed and reported become fatalities. As many as 88 to 90 per cent of such patients die. On the other hand, the morbidity rate is very low, 2.2 per 10,000 inhabitants of Makassar, and even less if the entire island is considered. Except among the family members of the ill, and other contacts, no carriers were found. Wells were often found infected. During the outbreak, the causative agent of the disease did not differ morphologically, biochemically or serologically from other El Tor vibrios which, around the Red Sea and in India, are not pathogenic or, at most, cause only insignificant bouts of diarrhea, without fatalities. The identity of vibrios with different human pathogenicity in two parts of the world is rather puzzling.

It is believed that this deviation warrants the use of different designations for the vibrio subtypes. It might be well to reserve the name *Vibrio El Tor* for the classic organism which was first isolated at the El Tor quarantine station by Gotschlich (1906) and then was seen repeatedly in the Near and Middle East. The vibrio causing paracholera in Sulawesi should be named *Vibrio de moor*, after the scientist who first reported it. This would be in accordance with such usage as *Vibrio metschnikovi*, *Vibrio koch*, etc.

A word should be said in favor of the nomen-

clature using paracholera or enteritis choleriformis El Tor or enteritis choleriformis de Moor-van Loghem. There are definite epidemiologic differences between so-called Asiatic cholera and the cholera-like disease appearing in Sulawesi. Thus, the name paracholera used by de Moor and van Loghem should be accepted to distinguish this disease from the classic cholera.

SUMMARY

An epidemic of paracholera in Sulawesi, which occurred in 1947 and 1948, is described, in connection with three previous outbreaks in which this disease was diagnosed on the same island.

No in vitro differences were found between the classic El Tor vibrio isolated first in Egypt, which is nonpathogenic, or of low human pathogenicity, and the Sulawesi strains which cause serious disease. It is suggested that the Sulawesi vibrios be designated as *Vibrio de Moor* after the scientist who first described them.

While paracholera resembles Asiatic cholera closely, it has some epidemiologic features which are different from those observed in classic cholera. Of these, sporadic occurrence with low morbidity but high mortality, and a generally static character, are the most outstanding.

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BOOK REVIEWS

Statistical Methodology in Malaria Work, by SATYA SWAROOP. World Health Organization/HS/58. Rev. 1, 2 September, 1957. 264 pp.

With the present world-wide emphasis on malaria eradication, it is highly important that workers involved in malaria research and control programs follow scientific methods free of personal bias and that they utilize standard procedures in experimental design and analysis. Satya Swaroop's book will be a real help to serious workers who wish to meet these standards. It is not a substitute for standard textbooks on statistics or malariometry but rather a guide to assist malariologists in adopting and utilizing appropriate statistical techniques and in establishing more effective collaboration with their statistical coworkers.

The book outlines the basic approaches for planning malaria investigations and for collecting, recording, tabulating, analyzing, and drawing conclusions from the data obtained. It gives a good introduction to statistical concepts, terminology, and methodology. Examples used cover a wide variety of the special problems involved in malaria eradication, including health and vital statistics, chemotherapy, and evaluation of insecticide resistance in anopheline vectors. Many tables and charts are included which permit the reading of results with a minimum of complicated calculations. The main chapters are: (2) Statistical Methods for Malaria Workers; (3) Vital and Health Statistics; (4) Malariometry; and (5) Adequacy of Sample Size. The appendices include helpful reference tables of logarithms, random sampling numbers, random allocation of treatments, table of chi-square, table of "t" and table of "F." The book is a mimeographed WHO publication.

It seems appropriate to make the following suggestions for improvement in the next edition: (1) include a subject index, (2) add a glossary of statistical terms, (3) give an annotated list of standard reference books on statistics and biometry, with comments on the specific usefulness of each, (4) include less information on vital statistics and more on indices such as are presented in Macdonald's recent book on Epidemiology and Control of Malaria, (5) simplify, and go into less detail on, some procedures such as probit analysis.

The book is one which every malariologist will want to have on his reference shelf.

A. D. HESS

A Handbook of Animal Physiology, by E. M. PANTELOURIS. 1st edition, 255 pages, ill. London, Bailliere, Tindall and Cox (Williams & Wilkins Co., U. S. Distributors), 1957, $6.25.

This is a basic general text on animal physiology giving an outline of the main facts and showing the wide scope of the subject. The book can be recommended as a first introduction. The material in it was selected mainly from topics used in examination papers in Britain for Biological, Agriculture and Veterinary degrees. Different views on unsolved problems are shown. A well selected reading list which contains many comprehensive articles, including experimental techniques, in easily accessible journals, is given. The book is well printed and illustrated by many diagrams and line drawings. A perusal showed only one error on Page 159 where "kilogram" should be used instead of "gramme" for the number of calories obtained from the complete combustion of fats or carbohydrates. The price for an introductory text of this size is somewhat high.

FREDERICK REISS

South African Snake Venoms and Antivenoms, by POUL AGERHOLM CHRISTENSEN, M.B., Dip. Bact., 1st edition, 129 pages, ill. Johannesburg, South Africa, The South African Institute for Medical Research, 1955:

This book is a useful source of information on certain of the zoological characteristics of the South African venomous snakes; and the nature, yield, stability, known pharmacological effects and toxicity of the venoms in experimental animals. There is an extensive, yet condensed, compilation of previously published and new data. The blood clotting mechanism, proteolytic action and enzyme content of the venoms described, and the physical and chemical properties of the poisons of certain South African species of Naja and the true vipers are given. Methods for sterilization, and detoxification of the venoms are described, and the literature is reviewed on various suggested medical uses of snake poisons.

The history of the development of an antivenin against South African venomous snakes is outlined, and the methods used for production, purification and concentration of the serum, animal assays of the neutralizing potency and determination of the cross-neutralizing action in vitro and in vivo are described in detail.

Information on the seasonal incidence and geographic distribution of snakebite in man and domestic animals in South Africa, and age distribution in man, body sites and other details are included. The mortality figures do not present a

true picture, since only the serum-treated victims are reported. The symptoms of bites by elapids, vipers and the boomslang are well described. The therapeutic methods advocated are not in complete agreement with thinking on the subject in accordance with latest experimental and clinical data available in the United States; *e.g.*, the author recommends use of the time-honored tourniquet, venesection and suction, although he does point out the dangers and disadvantages of such measures. He emphasizes the important principle that serum should be administered in adequate dosage; the smaller the body size of the victim, the larger the dose. The bibliography is comprehensive and the book is thoroughly indexed.

ELEANOR E. BUCKLEY

Clinical and Immunologic Aspects of Fungous Diseases, by J. WALTER WILSON, M.D., Los Angeles, California; Clinical Professor of Medicine, Dermatology, University of Southern California; Member and Director, American Board of Dermatology. 1st ed'n, 280 pp., Springfield, Illinois, Charles C Thomas, 1957, $6.75.

Those who know and admire J. Walter Wilson's charm and enthusiasm for medical mycology will enjoy this book, even if they may disagree with certain statements in it. This reviewer experiences both feelings and yet recommends the book highly, not only to the clinicians at whom it is aimed, but also to laboratory investigators and students first entering the field. A more stimulating (if at times controversial) treatise remains to be written on the facts and unsolved problems in fungous diseases.

Although the book's title suggests primary concern with immunology, still one of the greatest challenges in our understanding of the mycoses, the author's mind, as many know, sweeps the horizon and gives us the benefit of its far-reaching scan. Few students of mycology have had, as he admits, as inspiring an array of teachers and colleagues from whom to learn and on whom to test theories and dreams; few have done as much to disseminate information and enthusiasm. The readable, at times entertaining, style makes this book hard to put down unfinished. The author's disarming admission of liberties taken alerts the reader against accepting every statement as hard fact.

As the table of contents and introduction predict, coccidioidomycosis, Dr. Wilson's forte, supported by a wealth of clinical experience, is used as a model. In the first seven chapters he describes carefully and correctly practically all that is known about this disease; in the remaining fifteen chapters he draws parallels and suggests at times risky but nonetheless thought-provoking answers to open questions in many other fungous diseases. These include cutaneous as well as systemic mycoses, extending to infections produced by *Actinomyces* and *Nocardia*.

Because of its informative value and candid exposure of the author's thinking, this book should be of interest, and will suggest trails to explore, to epidemiologists, biochemists and physiologists, whether or not they are directly concerned with the diagnosis and therapy of fungous infections.

MARGARITA SILVA

Pharmacologic Principles of Medical Practice, by J. C. KRANTZ, JR. AND C. J. CARR. 4th edition, 1313 pages. Baltimore, Williams & Wilkins Company, 1958, $14.00.

This book will probably continue to serve as a useful synopsis for the medical student and a suitable textbook for students of nursing and pharmacy. However, as a treatise designed to present the principles of pharmacology this edition falls short of the mark. It still retains many of the characteristics of a materia medica and presents a rather cursory and uncritical compendium of modern principles of pharmacology. While it is true that the mechanisms underlying the pharmacologic action of most drugs have not been completely elucidated, many sound principles have been worked out. The physiologic disposition of drugs, knowledge of which is often imperative for the successful use of a compound, is given only minimal treatment by the authors. Some of the limited information included suggests a cursory critique; *e.g.*, although a specific method for the determination of isoniazid in body fluids has not yet been worked out, the authors discuss "plasma levels of isoniazid."

In view of the marked progress made toward control and eradication of malaria, the chapter on the chemotherapy of this disease seems particularly inadequate. The chapter on chemotherapy of tuberculosis is also disappointing from the standpoint of summarizing the recent advances made in the treatment of this important disease.

It is indeed unfortunate that the authors choose to continue their practice of using proprietary names in the description of some drugs. This policy is not only unbecoming a textbook; it may do the student a disservice if he fails to recognize the generic or official name on a certifying examination.

G. M. CONZELMAN, JR.

Schutzimpfungen, ed. by HEINZ SPIESS, Göttingen, with the collaboration of F. HANSEN, K. PETZELT, W. SCHÄFER, R. SCHINDLER, O. VIVELL, H. WIESENER, R. WOHLRAB. 356 + xvi

pp., with 41 illustrations, many in color. Stuttgart, George Thieme Verlag; U. S. A. and Canada, Intercontinental Medical Book Corporation, New York 16, New York, 1958. DM56.–, $13.35.

This well printed and illustrated book can not be said to make a special appeal to those solely or principally interested in tropical diseases, since only nine of its 356 text-pages deal with vaccination against yellow fever, cholera and plague, respectively, and, what is convenient for those who have but occasionally to advise intending travellers, also contain a scheme of the vaccinations required in the principal non-European countries. It is, however, of importance for readers in America that this section of the book, compiled by W. Schäfer, devotes its main attention to the prophylactic vaccination against yellow fever. The administration of this in combination with that of tetravaccine against typhoid, paratyphoid A and B and cholera as well as with smallpox inoculation is adequately described and due praise is bestowed upon the method as "the most effective preventive measure against yellow fever both for the protection of the individual and as a means to prevent epidemics."

Generally the book deals, after an adequate introductory section devoted to the principles of vaccination and immunity, with vaccination against diphtheria, tetanus, pertussis, scarlet-fever, tuberculosis, typhoid and paratyphoid, smallpox, rabies, poliomyelitis, influenza and adenovirus infections, measles, German measles, epidemic parotitis and hepatitis, and typhus. Additional chapters deal (a) with combined vaccinations, plans for vaccination and steps to avoid untoward reactions; and (b) with the legal aspects of the method (referring, however, only to the German laws and regulations).

Since all chapters are clearly written in a language which ought to create no difficulty even to readers who have not deeply delved into the intricacies of the German language, and are invariably well documented, it is rather invidious to single out any of them for special consideration. It deserves mention, however, that the book deals not only adequately with the longer known methods of vaccination, but also brings much useful information regarding more, or quite, recently introduced prophylactic methods, such as BCG vaccination and poliomyelitis vaccination, the value of both of which is admitted.

The problem of poliomyelitis vaccination is exhaustively discussed by Vivell who quotes no less than 300 references. Summarizing this evidence he states that

"According to the mass test in the US in 1954 and later experiences the result of active poliomyelitis vaccination practised thus far may be considered as favourable; the method is about 80% effective and practically innocuous."

As exemplified by the above statements, within the limits set forth this book can be thoroughly recommended.

R. POLLITZER

Non-Venereal Syphilis; A Sociological and Medical Study of Bejel, by ELLIS HERNDON HUDSON, M.D., DTM&H (London), F.A.C.P., Professor and Director of Health, Ohio University, Athens, Ohio. 1st edition, 203 pages, illustrated. Baltimore, Williams & Wilkins Company, 1958, $7.00 (U. S.).

It happens frequently in medicine (but not often enough) that an author's name becomes inextricably associated with a disease syndrome even if not in a nosological sense. And so it is with E. H. Hudson and the syndrome bejel, which virtually he alone brought into the consciousness of the medical world long before the publication of the present monograph.

Hudson began the study of bejel some 30 years ago as a young missionary physician in the Arab district of Deir-ez-Zor. After 10 years of work and study he returned to the United States, and engaged in various medical activities, but probably all the while brooded on his experience with bejel in relation to rapidly developing biological and clinical knowledge of syphilis and the other treponematoses. Fortunate man when, in 1950 he had the opportunity under the auspices of the World Health Organization, to return to the Euphrates Valley and, with all the perspective of the intervening years, to again study the treponematoses in this area of the world.

These brief biographical notations are made in order to suggest the flesh and blood that give body to the bare bones of the statistical material presented in this monograph. *Non-Venereal Syphilis*, indeed, is several small books in one. First and most importantly it is an authoritative description of the particular treponematosis to which the name bejel (pronounced bed'jel) has been given. Some 2,000 patients were studied and the clinical characteristics of the disease have been described with the aid of numerous well reproduced photographs.

In addition, interspersed throughout the 21 chapters are interesting sociological data on the Bedouin Arabs: family and sexual mores, the environmental situation in which bejel is endemic, and sociological differences between Bedouin peasants and Moslem and Christian towns-people.

Finally, coloring the whole are instructive comments on the history and biological interrelationshipe of the treponematosis group of diseases. From the accumulated evidence, much of it inevitably of an indirect nature, Hudson believes that the

original focus of the human treponematoses was probably in the rain-forest of Central Africa; that the "American" origin of syphilis is unsupported by existing evidence; that differentiation of the various treponemal syndromes, including syphilis, has come about largely through environmental rather than microbial genetic mechanisms.

Thus the author has not only made a lasting contribution to medical literature in his descriptions of the epidemiological and clinical features of bejel, but he has written a most entertaining book with philosophical overtones, in the best tradition of the student of the biology of human disease.

THOMAS B. TURNER

An International Nomenclature of Yaws Lesions (World Health Organization: Monograph Series No. 36) by C. J. HACKETT in cooperation with an International Group of Experts on Yaws and participants at the International Conference on Yaws Control, Enugu, Nigeria, 1955. English edition, 104 pages, illustrated. Geneva, W.H.O., 1958. $4.00.

When we define a disease for research, treatment, or disease-control purposes, and in simple communication about disease, common standards of diagnostic nomenclature are essential. In most diseases, nomenclature and classification of stages have posed problems. Often compromises have been developed, based on the best judgment available. These classification schemes serve certain needs, most often in terms of control or treatment, but are sometimes found insufficiently exact for research programs or other highly technical purposes. Experience has shown that a scheme of classification, prepared by those familiar with the disease, which is widely accepted and, more important, widely used, is invaluable in support of broad-scale public health programs.

The confusion of nomenclature on yaws, the wide variety of local terms, and until recently the lack of a clear-cut conception of how the disease evolves, have been handicaps in effecting control. This problem has been resolved by the World Health Organization, acting with advice and guidance provided by an international group of experts and the participants in the 1955 International Conference on Yaws Control at Enugu, Nigeria.

From the counsel of these groups, and from his own extensive clinical and research experience, Dr. C. J. Hackett, Medical Officer, Venereal Diseases and Treponematoses Section, WHO, and former Senior Fellow in Tropical Medicine of the Medical Research Council of Great Britain, has prepared what I consider to be the definitive monograph on international nomenclature of yaws lesions. This book, with beautiful clinical photographs accumulated over a 3-year period, should serve as an invaluable guide to all engaged in yaws control. One would expect such a definitive publication to provide a common basis in various countries for comparison of problems programs, and results.

Once again, the Venereal Disease and Treponematoses Section of the World Health Organization is to be complimented on developing and publishing a highly significant contribution to the literature in the field.

JOHN C. CUTLER

Intermediate Hosts of Schistosoma: African Biomphalaria and Bulinus, by G. MANDAHL-BARTH. English edition, 132 pages, ill. Geneva, W. H. O. Monograph Series No. 37, 1958. $4.00.

The African snails belonging to the genera *Biomphalaria* and *Bulinus* are of medical importance since they serve as intermediate hosts for human or animal schistosomiasis. In the literature authors have assigned these snails to a number of genera, e.g., *Planorbis, Planorbula, Afroplanorbis, Australorbis, Segmentina, Physa, Isidora,* etc., as well as *Biomphalaria* and *Bulinus*. In reference to species, however, the plethora of names given to these snails has reached a superfluous meaningless state as attested by everyone who is familiar with the synonomy. The main cause for the confusion of names has come from the fact that the shells of these freshwater snails are susceptible to alteration when subjected to different environments. They exhibit, therefore, a wide range of variation, both in size and shape. Large numbers of these ecological forms have been described as species, the description being based only on the shape or some other variable character of the shell.

Dr. Mandahl-Barth has redescribed the African species and subspecies of *Biomphalaria* and *Bulinus*. His studies have been based on fresh material which has been collected from a large number of localities throughout Africa. A splendid series of photographs of the shells accompany the descriptions. Anatomical dissections of nearly all of the species were made and specific determinations have been based on the dimensions and morphology of such organs as the verge, vergic sheath, preputium, and the radula. However, the dissections were made on specimens which had been preserved in a contracted state. This type of preservation causes some distortion of the soft organs, particularly the v. gina, uterus, and preputium. Discrepancies may be found, therefore, when specimens which have been killed in an extended position are dissected and compared with the descriptions and figures found in this publication.

A revision of the nomenclature of any group of animals or plants is generally based on the author's

concept of what constitutes a species. His ideas are influenced usually by a mosaic of principles and experiences and are, therefore, largely individualistic. For these reasons some malacologists or other scientists will not accept completely the conclusions presented in the publication by Dr. Mandahl-Barth. Some will probably regard certain subspecies as nothing more than ecophenotypes while others may elevate them to full specific rank. It is probable that when additional material has been collected and studied the nomenclature of these snails will become better stabilized.

This publication will be particularly useful to individuals interested in determining the species of intermediate hosts of schistosomiasis in Africa. It will certainly serve as a base line for future malacological studies of this important group of aquatic snails.

E. G. BERRY

Handbuch der Virusforschung—(Handbook of Virology), Vol. 4, edited by PROF. DR. C. HALLAUER, Bern and PROF. DR. K. F. MEYER, San Francisco. 688 pages, ill. Vienna, Springer-Verlag, 1958, $5.25.

Previous volumes of this Handbook, under the direction of its originators, Doerr and Hallauer, have presented the theoretical and practical aspects of virology as then known in an integrated and masterly fashion. The present editors, C. Hallauer and K. F. Meyer, have tried to adhere to the high traditions of the founders while attempting to meet modern requirements arising from vast expansions in newer knowledge. With the aid of a group of experts they have succeeded admirably. Following previous editions this one presents, in a general section, discussions of important modern technological and methodological advances, followed by a special section comprising discussions of specific viruses or groups of viruses in monographic style. With reviews of historical developments in the various fields and complete evaluations of the present state of knowledge in each, the volume serves very satisfactorily as a handbook for present use and a reference work for the future. The authorship is international and of international standing, and each author presents his respective section in his own language: *Genetics of animal viruses*, F. M. Burnet; *Genetics of bacteriophages*, P. Fredericq; *Viruses of insects*, G. H. Bergold; *Arthropods as vectors and reservoirs of phytopathogenic viruses*, K. M. Smith; *Arthropods as vectors and reservoirs of animal pathogenic viruses*, W. C. Reeves; *The study of viruses pathogenic for man by means of tissue cultures*, W. Klöne; *The seasonal encephalitides*, P. Lépine and P. Goube de Laforest; *The hoof-and-mouth virus*, H. Röhrer and G. Pyl; *Columbia SK group of viruses*, C. W. Jungeblut; *The virus of poliomyelitis*, S. Gard; *The Coxsackie virus group*, H. Löffler.

The book contains many illustrative materials of excellent quality: graphs, line drawings, diagrams, photographs, electronographs, etc. Each author has included an extensive, even exhaustive, bibliography, some comprising over 600 titles. The sections on tissue culture, on arthropods as vectors both of phytopathogenic and zoopathogenic viruses and the related section on viruses of insects (entomopathogenic (?) viruses) and the sections on genetic aspects of bacteriophages and of animal viruses appealed most to this reviewer but the sections on seasonal viruses, (with explanatory footnote *re.*, "seasonal" by the authors), and on polio and related viruses will be of greatest interest to experts on those agents.

This book is a mine of information. In books, as in mines, one seldom finds everything he wants and sometimes finds things he didn't expect. This is because books (and reviews) are written by human beings. This particular book is an outstanding contribution and an ornament to the virological literature; a necessity on the shelf of every microbiologist.

MARTIN FROBISHER

Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death, WORLD HEALTH ORGANIZATION, Vol. 1. xli + 393 pages, 1957, $6.75 (2-vol. set price).

The system used internationally to classify causes of death and of morbidity has now been revised for the seventh time since its inception in 1893. This is the second time the classification has been published by the World Health Organization. The sixth revision, the first released under its direction, represented a sweeping reorganization of previous lists to create a combined classification of disease conditions, injuries, and causes of death. The present edition retains the structure and general detail of the sixth, with a few minor modifications found desirable in the years of its use. In addition, subdivisions according to detailed anatomical site were added to the section on neoplasms.

Use of the classification makes it possible for comparable statistics on diseases and causes of death to be produced in various hospitals, health agencies, and vital statistics offices in this and in other countries. These statistics depend on the method of selecting the cause of illness or death that is shown in statistical tabulations, as well as on the classification scheme itself. The Manual recognizes this problem and provides a set of rules for selecting the condition to be classified and examples of their application. Supplementary classifications for causes of stillbirth and for impairments are also given.

The seventh revision, prepared in 1955 and released for use in 1958, marks a change in the decennial revision cycle. Earlier revisions were generally released in the year ending with "9" or "0." The year ending in "0" or "1" is also the year in which population censuses are taken in many countries. The advance of the revision date was designed to permit countries to gain experience with the new revision before undertaking mortality studies related to the census year.

The alphabetical index, the second volume of the Manual, provides a convenient method for locating the classification number of any disease condition.

LILLIAN GURALNICK

BOOKS RECEIVED

Morphologische, Biologische u. Serologische Eigenschaften der Bartonelle, by Priv-Doz. Dr. Reinhard Wigand mit einem Geleitwort v. Prof. Dr. E. G. Nauk, Bernhard-Nocht Ins't f. Schiffs-u.Tropenkeiten, Hamburg. 1st edition, 95 pages, illustrated. Stuttgart, Georg Thieme Verlag, 1958. DM 7.80, $1.85.*

Antisera, Toxoids, Vaccines & Tuberculins, by H. J. Parish, M.D., F.R.C.P.E., D.P.H., Clinical Research Director, Wellcome Foundation, London. 4th edition, 256 + x pages, illustrated. Edinburgh and London, E. & S. Livingstone, Ltd. (Williams and Wilkins, exclusive U. S. agents), 1958. $7.00.

Leptospirosis in Man and Animals, by J. M. Alston, M.D., F.A.C.P., Edin., J. C. Broom, O.B.E., M.D., and C. J. A. Doughty, B.A., with Foreword by Sir Samuel Bedson, M.D., D.Sc., F.R.C.P., F.R.S. 367 + xii pages, illustrated. Edinburgh and London, E. &. S. Livingstone, Ltd., 1958, $8.50.

The Psychology of Medical Practice, by Marc H. Hollender, M.D. 276 pages. Philadelphia, W. B. Saunders Company, 1958, $6.50.*

World Atlas of Epidemic Diseases, by E. Rodenwalt and H. J. Jusatz, Editors. Heidelberger Akadamie der Wissenschaften. Part III, No. 2 32 pages, 10 maps. Hamburg, Falk Verlag, 1958.

Industry and Tropical Health III. Proceedings 3rd Conference Industrial Health Council of Tropical Health, by various authors. 261 pages, illustrated. Boston, Harvard School of Public Health, 1957. $10.00 and $5.00 (Prof.)*

Veterinary Protozoology, by U. F. Richardson, B.Sc., M.R.C.V.S., & S. B. Kendall, B.Sc., Ph.D., A.R.C.S., M.R.C.V.S. 2nd edition, 260 pages, illustrated. Edinburgh and London, Oliver and Boyd (The MacMillan Company, N. Y., U. S. distributors), 1957. $4.50.*

Annual Review of Entomology, by various authors. 1958, Volume 3, 520 pages. Palo Alto, California, Annual Reviews, Inc., 1958. $7.00.*

Wissenschafliche Zeitschrift der Humboldt-Universität zu Berlin, by various authors. Yr. 6, 1956/7, pp. 449–602, illustrated. Leipzig, Leipziger Druckhaus, 1958.*

Bulletins et Mémoires de l'École Nationale de Médécin et de Pharmacie de Dakar, by various authors. Volume 5, 368 pages, illustrated. Paris, Expansion Scientifique Francaise, Editor, 1957.*

Quadriennial Report of the Director of the Pan American Sanitary Bureau Regional Office of the World Health Organization, 1954–1957, and the 1957 Annual Report, by Fred L. Soper, M.D., Director, Pan American Sanitary Bureau. 238 pages, illustrated. Washington, D. C., Pan American Sanitary Bureau, 1958.

Author Publisher Printer Complex, by Robert S. Gill. 3rd edition, 135 pages, illustrated. Baltimore, Williams and Wilkins Company, 1958, $2.25.

International Standards for Drinking Water, by World Health Organization Study Group on International Standards of Drinking-Water Quality. 152 pages, illustrated. Geneva, World Health Organization, 1958. $4.00, £1, Sw. fr. 12.

Progress in Medical Virology, by E. Berger (Basel) and J. L. Melnick (Houston, Texas) Editors. Volume 1, 304 + vii pages, illustrated. New York, Hafner Publishing Company, 1958. $11.50.*

The National Foundation for Infantile Paralysis. Annual Report, 1957, by Basil O'Connor, President. 83 pages, illustrated. New York, National Foundation for Infantile Paralysis, 1958.*

Official Documents of the Pan American Sanitary Bureau No. 22. X Meeting—Directing Council of the Pan American Sanitary Organization. IX Meeting—Regional Committee of the World Health Organization for the Americas, by Pan American Sanitary Bureau Regional

* Referred for review to "Tropical Medicine and Hygiene News" Published by **The American Society of Tropical Medicine and Hygiene**; Editor, Dr. Louis J. Olivier, National Institutes of Health, Bethesda 14, Maryland, U. S. A.

Office of the World Health Organization, Washington, D. C. 295 + viii pages. Washington, D. C., Pan American Sanitary Bureau, 1958.

Bulletin d'Information du Ministere de la Santé Publique et de la Population. Trimestriel, 1957, No. 4. by various authors. 93 pages. Paris, Ministere de la Sante, 1958. 400 fr. (1,200 per year).

A Doctor Speaks His Mind, by Roger I. Lee, M.D. 120 pages, illustrated. Boston, Little, Brown and Company, 1958. $3.00.

World Health Organization Annual Epidemiological and Vital Statistics. 1955, W. H. O. 699 pages. Geneva, W. H. O., 1958. £3, $12.00, Sw. Fr. 36.

Science, the News and the Public. A Report of the National Association of Science Writers, Inc., by Hillier Krieghbaum, Associate Professor of Journalism, N. Y. U. 43 pages, ill. New York City, New York University Press, 1958.

Samuel Taylor Darling, by Gerald L. Baum, M.D. 35 pages, ill. 1958.

Immunization Information for International Travel, United States Dep't. of Health, Education and Welfare. June, 1958 Revision, 71 pages, with maps. Washington, D. C., Government Printing Office, 1958. $0.30.

Insecticide Resistance in Arthropods, by A. W. A. Brown, Ph.D. 240 pages, ill. Geneva, World Health Organization (Columbia Univ. Press, N. Y., Distribution Agent in U. S.), 1958. $5.00.

NOTICE TO CONTRIBUTORS

The Journal welcomes papers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and arthropod-borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted articles which raise no question of scientific priority but for which immediate publication seems important will be inserted in the next available issue of the journal if the author or sponsoring institution will pay the cost, estimate of which may be obtained from the editor. The insertion of such articles does not affect publication of other manuscripts awaiting their turn, since the inserted articles constitute additional pages in the volume without additional cost to the subscriber.

Publication will be facilitated if the following suggestions are followed:

1. *Manuscripts* should be typewritten in *English* using *double spacing* on *one side* only of 8½ x 11 inch paper and submitted *in duplicate*. Double-spacing should be used throughout the manuscript including the list of references, footnotes, and quotations.

2. The author should supply, on a separate sheet, a short title not over 50 letters for use as a running head. Also on separate sheets type (a) the authors' names and affiliations; (b) tabular matter; (c) case histories; (d) quotations; (e) formulas and other subsidiary matter in the text; (f) footnotes; (g) references; (h) legends for illustrations; (i) displayed formulas occupying more than 1 line of type, even if using the same size type as the main text.

3. Legends should be separate and not attached to, or written on, the illustration copy.

4. Positions for tables and figures in the text may be indicated on the margin of the text pages.

5. All papers should end with a brief summary.

6. *Tables* should be typed on separate sheets and be serially numbered in Arabic numerals.

7. *Figures* and *photographs* should be protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be made with India ink and submitted on white (not cross-section) paper. Colored illustrations can be reproduced only at the expense of the author.

8. References should be cited by consecutive numbers in the text, and so numbered and listed in the list of references at the end of the article, without regard to alphabetical arrangement. References should be written as follows:

> Reed, L. J., and Muench, H., 1938. A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.*, 27: 493–497.

9. *Proprietary names* of drugs or chemicals registered by commercial pharmaceutical firms may not be used in the text if there is a generic name available in the U. S. Pharmacopoeia, New and Nonofficial Remedies of the A. M. A., or the latest editions of standard medical dictionaries. In certain cases where the generic name is relatively unknown, the registered name may follow it in parentheses in the title, at the first citation in the text, and in the conclusions.

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